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ERRATA.

- Page 150, line 2 of *References*, for "Engnr" read "Engng"
- Page 153, line 2, after "fly survey" insert "on areas in which *Glossina medicorum*, *G. fusca* and *G. longipalpis* may occur"
- Page 154, line 2, for "yet a search was made" read "yet this search had been made"
- Page 289, 5 lines from end, for "dichlorodiethyltrichloroethane" read "dichlorodiphenyltrichloroethane"
- Page 289, 2 lines from end, for "1,2,3, . . . 8," read "2,3, . . . 8,"
- Page 511, line 16, for "gravic" read "gravid"
- Page 514, Table II (4), Col. 2, for "IV Sprayed" read "IV Dipped"
- Page 575, line 17, for "15" read "(B) 15"
- Page 594, line 12, & page 598, line 4, for "mercurous bichloride" read "mercurous chloride"
- Page 598, line 9, for "six" read "five"
- Page 369, line 18, for "mm." read "cm."

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FACTORS INFLUENCING THE ACTION OF DUST INSECTICIDES.

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The insecticidal preparations which first came into use were almost certainly powders and the use of dusts has remained a popular and important method of controlling pests ever since. In more recent years liquid preparations have been greatly developed but they have never displaced dusts, especially for large scale use, such as on cotton crops, forest trees and mosquito breeding grounds.

The literature on dust insecticides is extensive, especially on practical application, but far less information is available about the precise mode of action of the dusts and it is only comparatively recently that the subject has been studied in any detail.

The field tests with dusts yield very little information about the way in which they act since the experimental situation is so complex and uncontrolled. In the field two main groups of factors are concerned : those associated with the formation, settling and distribution of the dust cloud and another group concerned with the relationship between the insect and the dust. It is convenient when investigating the action of dusts to separate these two groups of factors and it is only the latter group which has been studied in the present investigation.

Theory.

In order to understand the mode of action of a dust insecticide, it is first necessary to study the properties of the dust and the insects on which it is to exert its action. In a later section these subjects are discussed and the properties of both the insects and the dusts have been described. The matter can, however, be viewed with advantage first from the theoretical standpoint as will be done in the present section.

Specific gravity.

When the force of gravity acts on a dust particle in such a way as to tend to remove it from the position on the insect to which it is adhering, the probability of the separation occurring will be greater when the density is high since, all other things being equal, the weight, and so the strain on the interfacial forces of adhesion, will then be greater.

In practice variations in specific gravity do not usually occur independently of changes in chemical composition which may also influence the forces of adhesion and a satisfactory practical demonstration of the part played by gravity is not easily realised.

Bulk density.

Apart from the rôle played by the specific gravity of the constituent material in determining the bulk density of a powder, this property varies enormously with the volume of the interparticular spaces commonly termed "voids". (When the particles themselves are porous, as in diatomaceous earths, this will also affect the bulk density.) The percentage of a bulk of a powder represented by voids is not directly related to particle size but between certain limits there is a tendency for the percentage of voids to be highest in the finest powders. This relationship is however not invariably true since if different sized particles are present the voids between the larger particles, which would otherwise be empty, will become filled and so the bulk density value will be higher than it would be in their absence (Roller, 1930 ; Borchers & May, 1935.) The bulk density is low too when the particles composing the powder have a very irregular shape and it has been found (Roller, 1930) that particle shape greatly influences the degree of packing. If at the same time as being irregular the individual particles are light (due to internal spaces) the tendency for them to pack closely will be at a minimum and the bulk at a maximum. These latter conditions are found in a diatomaceous earth.

When toxic dusts, made by dispersing the poisons in non-toxic carriers, are considered it can be seen that, if made up on a weight basis, those which contain the most voluminous carriers will contain the least toxic material per unit volume. In general terms the weight/volume concentration decreases as the voluminosity of the carrier dust increases and, from analogy with solutions, it might be expected that when the concentration of poison is lower then its effect would be less. However,

with particulate material, which probably only makes contact with an insect at isolated points, and then to an extent and degree of closeness which may be determined by other properties of the dusts, it would not be surprising if the voluminosity effect was not apparent. Again, since a low bulk density, *i.e.* a high voluminosity, tends to be associated with low particle size and fine particles adhere to surfaces more strongly than coarse, it might well be expected that the superior adherence, if this comes into play, would counterbalance the dilution effect of high voluminosity.

Particle size.

Various methods have been used to determine the particle size ranges of dusts which pass through the finest sieves and, therefore, come within what is called the "sub-sieve size range". The measurement may be effected by direct microscopic examination but this is a tedious process and is often replaced by some more or less indirect procedure such as sedimentation analysis, the light extinction method or the air-permeability method. From the extensive literature it is evident that the subject presents many difficulties and for further information references should be made to publications by Dalla Valle (1943), Skinner & others (1944), Amer. Soc. Test. Mat. (1941), Heywood (1938, 1947) and the lists of papers given therein.

The individual particles in a powder are of irregular shape, and it is difficult to know what dimensions to measure to express their sizes. They also frequently adhere to one another so closely and intimately that it is not easy to complete the dispersal of the aggregates without fragmenting the actual particles. It may be seen, therefore, that the dividing line between particles and aggregates is not very sharp and that in a given experimental situation the acting particles may be aggregates. For the purpose of relating insecticidal efficiency to particle size, it is important to ensure that the state of aggregation of the dust when it comes into contact with the insect is approximately the same as when the particle size distribution determination is made. When making comparative particle size measurements between dusts, it is necessary to select and keep to some definite system of preparing the sample and measuring the particles. In this way repeatable particle size distribution curves for the particular set of circumstances chosen may be constructed, but it cannot be assumed that data obtained on samples dispersed in a liquid-dispersing medium will be applicable to a dust cloud of the same material.

The particle size of a dust may influence its insecticidal properties in several ways according to the nature of its action on the insect; in the case of abrasive dusts or contact poisons it will exert an important influence on the quantity adhering. It has been found by experiment that particles of diameter above 15μ adhered poorly both to each other and to the insect, adherence improved progressively below a value of about 10μ diameter; at 5μ the particles adhered well to the insect while at about 2μ or under, aggregation was considerable (Alexander & others, 1944b).

According to the circumstances of application adherence may or may not be an important factor. In the presence of an excess of dust it is of no consequence, but even so the size-range of the particles may be important because it has been shown that only fine powders cause the insects to become desiccated (Chiu, 1939; this paper p. 27). Wigglesworth (1947) has shown that much of the abrasion caused by dusts takes place at the articulations and junctions of the body segments and the ineffectiveness of large particles is possibly connected with their failure to get under the margins of the articulating surfaces. There may, however, also be a lower limit of size below which effectiveness falls off for some reason. Parkin (1944) found that quartz particles of average diameter 1.8μ were more effective than those averaging 0.5μ .

Particles above a certain size obviously could not be eaten by insects and it has in fact been shown (Shipitzina, 1935) that the maximum size of quartz particles consumed was greater in the case of a fourth-stage mosquito larva than a first-stage. The entry of dusts into the spiracles will also be limited by particle size as has been shown by

Hamilton (1937) using cuprous cyanide dust with motionless and flying locusts and by Webb (1945a & b) regarding the entry of rotenone dust into the spiracle of the ked, *Melophagus ovinus* (L.).

Particle size does not, however, only influence access of the dust to the site of action, but in the case of a toxic dust the more finely divided material, owing to its larger surface, exerts a more rapid action than an equal weight of material of larger particle size. This observation has been shown to be true for contact poisons, such as pyrethrum dusts acting on Aphids (Smith, 1936) and stomach poisons, for example, lead arsenate or cryolite acting on the honey bee (Bertholf & Pilsen, 1941) and paris green acting on the Mexican bean beetle (McGovran & others 1940). These results are hardly surprising since only a small percentage of the material composing a large particle is exposed to the surfaces with which it makes contact.

Subdivision would facilitate absorption and action of the insecticide if the divided material all reached a site of effective action, but if absorption only occurred on relatively restricted areas, it might be preferable to have large particles each of which would constitute a lethal dose, if it chanced to contact one of these areas.

When the same material in two distinct particle size ranges is dusted on to surfaces, the weight of material which adheres in each case will be determined by the numbers and sizes of the particles present. It is possible that relatively few of the coarser particles lodged mechanically among surface irregularities might represent more material than numerous smaller particles adhering by interfacial forces to the general surface. However, as has just been pointed out, the coarser particles with their small surface are not likely to be very effective. In assessing the probable effect of a toxic dust it is always necessary to take into account these two factors—quantity and particle size—but even so it may be difficult to forecast the results since the influence of aggregates and the rate of absorption at the various sites of lodgement remain unknown.

Particle shape.

Because of the existence of aggregates, the particle shape, like the effective particle size, of a dust is often difficult to determine and the considerations set out under the section on size are also applicable here.

Theoretically it seems probable that the shape of dust particles will influence their mutual adherence and their adherence to insects, for example flat plate-like particles with large areas of contact would be expected to adhere better than spheres. Two effects might be concerned in this adherence—one acting by the interlocking of gross surface irregularities, the other by the close appositions of smooth surfaces. From the standpoint of biological action a sharp irregular particle would be most likely to abrade the integument, while every particle making a large area of intimate contact should be efficient in adhering and in passing on toxic material to the insect.

If the relationship of a dust to a surface is further considered, it can be seen that it may appear to cover a surface without having a very large area of close contact with it. This would be the case when spherical particles rested on a plane surface. The coverage would be estimated in terms of the cross sectional area at the diameter horizontal to the surface but the close contact area would be little more than a number of points. If the spherical particles were themselves inert, but coated with a thin layer of toxic material, very little poison might be transferred to the surfaces on which they rested unless some additional process, such as surface diffusion, or spread of a mobile solvent from the substrate over the particle surface, took place. As the particle shape passed from octahedral to hexahedral forms it would reach in the cube an area of contact equal to the cross sectional area and so the covering power and the area of active contact would have become equal at one-sixth of the total particle surface area.

From the point where the cube has been reached, the ratio of contact area to total surface area can be further increased by splitting the cube into plate-like particles until a ratio of nearly one half is reached in a flake of infinitesimal thickness. Nothing would be added to either the actual covering power or contact area on a flat surface by breaking these plate-like particles at right angles to their flat sides though optical effects might make it appear so. Subdivision in this case, although it adds slightly to the surface area of the dust, does not increase its covering powers or contact area, and will not do so if continued in the same vertical plane until the particles no longer rest on their largest face and move to a new position of stable equilibrium. The situation, however, may be different on curved or irregular surfaces where fragmenting plates vertically may help a limited quantity of dust to make intimate contact by enabling the particles to follow the contours in a way that larger rigid particles could not do. In view of the magnitude of the particles and the shape of the surfaces usually involved in insecticidal action, it seems probable that subdivision would always increase the area of close contact. In practice, of course, the dust particles may not orientate on the insects in the optimum position to exert their effects—in this connection it has been shown that on glass plates particles of talc may adhere by their edges (Voelkel, 1929).

Specific Surface.

The specific surface of a powder is so closely related to the size and shape of the particles that many of the theories associated with it have already been discussed.

In extent the surface area of a powder may be equivalent to that of the smooth surfaces of the particles or it may be much greater than this if the particles are fissured, finely porous, or hollow as in the case of the diatoms which compose certain natural earths.

From the point of view of insecticides, the chief possible importance of the specific surface would seem to be in connection with insecticidal powders made by distributing a toxic ingredient in solution over the surface of a non-toxic carrier and subsequently evaporating the solvent. In this way the poison, say DDT, would be either distributed over the surface of the individual particles or perhaps deposited in the spaces between them. In the former case the deposit per sq. cm. of carrier surface would decrease with an increase in the specific surface of the carrier and this might be regarded as equivalent to dilution. The magnitude of the effect for the carriers used in the present investigation is shown in Table I from which it can be seen that the deposit might vary between 0.9 and 83 μ g. per sq. cm. of powder surface. These figures are quite low compared with those necessary to render a surface insecticidal with a short period of contact (Busvine & Barnes, 1947).

TABLE I.

Carrier Dust	Specific surface by air permeability sq. cm./g.	DDT μ g./sq. cm.
Calcium carbonate ...	600	83.0
Talc ...	6,280	7.9
Slate ...	7,590	6.6
Stockalite-Kaolin ...	23,210	2.6
Almicide-Alumina ...	29,460	1.7
Neosyl-Silica ...	52,790	0.9

Deposit in μ g. per sq. cm. for a 5 per cent. w/w preparation of DDT on various carriers. The DDT was applied to the carrier from a volatile solvent and it was assumed that it would all coat the surface of the particles of the carriers and that none would be deposited independently in the voids.

In view of the wide variation in the deposit per unit area calculated for the various carriers, it might be supposed that those with the smaller specific surface and consequently the higher deposit would be most toxic. Even if the DDT or other deposited insecticide did not mainly coat the particles of the carriers, it would be more dispersed in the powders with the higher specific surface since they are usually more voluminous and this might also be viewed as a dilution effect. However, other factors are involved and this expectation may not be fulfilled in practice.

Hardness.

The part which the hardness of a dust played in producing the death of insects by desiccation has been investigated by Alexander & others (1944b). They showed that dusts which ranked high on Moh's scale of hardness were usually more effective in causing desiccation and death than other dusts which were lower on the scale. There were, however, certain anomalous results and some soft materials like galena, magnesite and zinc-blende were more effective than their hardness suggested. Furthermore it was found that the effectiveness of a hard material was reduced by treating it in a manner calculated to wear away its sharp edges.

Wigglesworth (1944, 1945) has shown that the desiccation, and consequently death, that occurs among insects which have been dusted, results from the damage done to the cuticle, so that it is not surprising to find that the hardness and sharpness of the particles are important properties. He also showed that, in certain cases, the abraded cuticle was more permeable to insecticides and it might be postulated that an abrasive carrier would facilitate the entry of insecticides under practical conditions. This hypothesis is discussed later.

Moisture relations.

When a dust is exposed to a moist atmosphere, an adjustment in its moisture content occurs until a value which is in equilibrium with that of the surrounding air is eventually reached (Wilson & Fuwa, 1922). The total moisture content of the dust exposed to air of relative humidities between 0 and 100 per cent. may never exceed a fraction of 1 per cent., but hygroscopic materials absorb larger quantities of moisture and the variation of the equilibrium moisture content with changes in humidity will be correspondingly great.

The moisture content of a dust may have a large influence on its insecticidal action. For example, if an insect comes into contact with a quantity of a desiccating dust it may be killed by direct water loss, while in the case of toxic dusts the desiccating effect may be added to the toxic effect and so give the impression that the toxic material itself is more active in certain carriers than in others. The desiccating effect should be most marked in the case of abrasive carriers with high moisture absorbing capacities, e.g. alumina and silica and of least importance in the absence of strong abrasive or absorptive properties, e.g. talc and calcium carbonate.

When the insect is not exposed to an excess of dust, the moisture content of the dust may yet influence its insecticidal action, through the effect it may be expected to have on the quantity of dust adhering to the insect or the substratum on which it lives.

Finally, the humidities of the dust and the atmosphere, between which an equilibrium will be established, are known to influence the formation of dust clouds through the effect they have on the mutual adhesion of the particles and the electrostatic properties of the cloud. It has been shown (Wilson & others, 1944) that at low humidities marked electrostatic charges are developed by the dust particle due to the friction which they experience in the dusting machine, but in atmospheres at relative humidities much above 50 per cent. these charges are quickly lost. At the lower humidities it seems possible that the particles may still carry a charge when they come into contact with the insect and this may well influence their deposition and

orientation, especially as it has been shown that insects may also carry charges localised in certain areas (Heuschmann, 1929).

Electrical properties.

Dust particles often carry charges which may be held very tenaciously especially in dry atmospheres. They may perhaps be of the greatest importance in connection with the formation of dust clouds and the process of ejecting the dust from a dusting machine with compressed air often leads to the development of a considerable charge (Whitman, 1926; MacLeod & Smith, 1943; Wilson & others, 1944). Apart from its effect on the behaviour of a dust cloud, the charge on the particle may cause it to be either attracted to the insect, or repelled from it, according to whether the charges on each are of opposite or of similar sign. Once the particle has landed, electrostatic forces may help to hold it in position.

List and General Description of the Non-Toxic Dusts used as Carriers.

Samples of a large variety of dusts were obtained in preparation for the experiments but after a preliminary examination most of these were rejected. The dusts which were retained had well differentiated physical properties and consequently might be expected to show interesting differences. Certain other dusts were used in the tests for special purposes. These included materials which appeared to have similar physical properties but yet differed in their insecticidal effects (e.g. various carbon blacks) or in some other way showed anomalous properties.

"Almicide".

Almicide is an artificially prepared alumina-aluminium oxide Al_2O_3 . It is very abrasive and hygroscopic. Under the microscope it can be seen to consist of large thin, translucent flakes covered with a mosaic of cracks: these flakes may be up to about 120μ long but they quite readily disperse into particles about $0.5\text{--}2\mu$ in diameter.

"Stockalite".

Stockalite is colloidal Kaolin ($\text{H}_4\text{Al}_2\text{Si}_2\text{O}_9$). It consists of small, irregular, translucent particles mainly $1\text{--}2\mu$ diameter, with some larger particles up to about 10μ diameter. It is neither markedly abrasive nor hygroscopic.

Slate Dust.

Slate is an aluminium silicate (56 per cent. silica, 21 per cent. alumina, 10 per cent. mixed iron oxides, etc.). The individual particles are flaky and angular the thickness being $0.1\text{--}0.25$ of the breadth and the "99 % passing 300 mesh Tyler" grade used contains particles of all sizes up to about 40μ in diameter. Slate is moderately abrasive but the water absorption capacity is low.

Talc.

Talc is hydrated magnesium silicate ($\text{H}_2\text{Mg}_3\text{Si}_4\text{O}_{12}$). The samples employed consisted mainly of thin plates with angular or rounded margins of all sizes up to about 80μ in diameter although there were also a few very elongated fibrous particles present. Talc is only slightly abrasive and absorbs little moisture.

"Neosyl".

Neosyl is a form of silica (SiO_2). It consists of very fine particles which are all below 1μ in diameter. It readily forms aggregates. Neosyl has good abrasive properties and it is also very hygroscopic.

"Sil-o-cel".

Like Neosyl, Sil-o-cel is also largely silica, SiO_2 , but unlike Neosyl it is a diatomaceous earth and consists largely of porous diatom shells of a great variety of shapes.

In a few cases the particles may be as much as 50μ long and 4μ wide but the maximum dimension does not usually exceed 10μ . The material is fairly abrasive and hygroscopic.

Calcium Carbonate.

The sample used was prepared by precipitation and consisted of well-defined crystals mostly about 10μ in diameter. There were very few crystals much smaller than this diameter and hardly any which exceeded 20μ .

"Carborundum".

Carborundum is silicon carbide and was chosen for these experiments because it is prepared commercially in carefully graded particle size ranges. The material is also dark in colour and therefore easily visible amongst insect tissue. The particles are sharply angular and range from a minimum of 1μ long in the finest "700 grade" to a maximum of about 200μ long in the coarsest "120 grade". The material is, of course, hard and abrasive but not hygroscopic.

Carbon Blacks.

Carbon in the form of lamp and acetylene blacks was obtained because, as pointed out by Alexander & others (1944b), they vary in their capacity to produce death through desiccation in an unaccountable manner. The particles are small, about 1μ in diameter, and show a marked tendency to form aggregates.

Glass.

Powdered glass in the form of sharply angular, thin, transparent flakes was used together with the same material formed into spheres by heat treatment. Both materials contained particles from 2 to 50μ in diameter, with the majority 2– 20μ , but—probably owing to loss of some of the fine material in heat treatment—the spherical material was slightly coarser. The abrasive properties were poor and glass was, of course, not appreciably hygroscopic.

Methods of investigating the Physical Properties of Dusts and Data on the Dusts employed.

Whether a dust exerts its insecticidal effect mechanically by abrading or in some way damaging the cuticle and perhaps the gut of the insect, or chemically through its toxic nature, it is evident that the chemical and physical properties of the dust will be important guides to its biological action.

It may, for example, be shown that small particles adhere and abrade more readily than large particles and that hard angular particles are more abrasive than soft or rounded ones. These are but a few of the relationships between physical properties and biological action which may be postulated if not always demonstrated.

In view of the foregoing remarks the properties of a variety of dusts were determined as a preliminary to studying their mode of action in insecticides and, where it has been found possible to do so, the relationships between the physical properties and the insecticidal effects have been illustrated by the experiments recorded on p. 25.

Specific gravity.

The specific gravities were determined on samples of the dusts which had been thoroughly dried in an oven at 105°C . About 0.5 g., or rather less than a quarter of a 10 cc. gravity bottle full of dust, was taken and covered with dried and filtered odourless kerosene so that the bottle was about half full. It was then placed in a desiccator under reduced pressure (16 mm. Hg.) for 30 minutes or until no more air bubbles rose from the dust. Afterwards the bottle was completely filled with kerosene and transferred to a water bath at 25°C . for one hour and subsequently reweighed.

The results obtained will be found in Table II.

Bulk density (loose bulking value).

The bulk density value expressed in cc. g. measures the volume of a given weight of any dust under a set of arbitrarily chosen conditions. The value is not an absolute one and depends on such circumstances as the way in which the powder is filled into the measuring vessel and the shape of the vessel used.

Two methods were employed in order to determine the bulk density.

(a) The large-scale method :

This method has been described by Goodhue (1937) and was used with the dusts as received. A light metal container $2\frac{3}{4}$ in. high and 2 in. in internal diameter was weighed and filled with dust which had previously been sieved through a 30 mesh sieve conforming to BSS 481. When the container was filled to overflowing without any tapping or settling it was levelled off with the stroke of a knife or spatula and then reweighed. The test was duplicated on each sample.

(b) The small scale method :

A small scale method has been described by Roller (1930). In this case a tube closed at one end about 4.5 mm. in internal diameter and graduated at 36 mm. was employed. (These figures are not critical but the length to the graduation mark should be at least six times the diameter.) The actual volume was 0.649 cc. as determined by weighing the volume of water necessary to fill it up to the calibration mark.

The dust, which had been conditioned in an atmosphere at 25 C. and 60 per cent. relative humidity, was sieved through a 2 in. diameter 30-mesh (BSS 481) sieve into a funnel which was attached to the graduated tube by a length of rubber tubing. It was then shaken into the tube by giving the bottom fifty taps on a hard rubber bung. When the dust filled the tube slightly above the mark the funnel was disconnected and the tube was given a further fifty taps. Any surplus dust above the mark was loosened with a pin and gently emptied out. Duplicate determinations which agreed within 5 per cent. were made.

The chief difference between the two methods was that in the second the dusts were compacted by tapping and the bulk density figures were therefore higher, the maximum difference being found in those dusts where the voids tended to be large.

The results obtained by both methods are shown in Table II.

Particle size.

No precise studies of the particle size distribution of the dusts were carried out but each dust was examined either in a solution of the proprietary wetting agent "Teepol" or in oleic acid which dispersed the aggregates. The sizes of the particles present were noted in a general way and the conclusions were related to the information gained from specific surface determinations, Table II.

Particle shape.

The same procedure was followed as in the examination of particle size. Sometimes the dust was examined dry by reflected light.

Specific surface.

The total surface area of all the particles in a dust when expressed in sq. cms. per gram is known as the "specific surface".

Several methods, depending on different principles, have been used for determining the specific surface of powders. Some of the more commonly used are the air permeability method (Lee & Nurse, 1939, 1947); the light extinction method (Skinner & others, 1944) and the absorption method (Gregg, 1947). The first method, following the procedure of Lee and Nurse (1939), was applied here. In connection

with all these methods it should be noted that while most methods yielded accurate relative values on different samples of the same materials, the results were less valid when comparisons were made between different substances.

For details of the permeability method employed references should be made to the original papers (Lee & Nurse, 1939, 1947). In brief, the procedure consisted of measuring the resistance of a specially compacted bed of powder to the passage of air in terms of the pressure difference between two kerosene manometers.

The results obtained are shown in Table II.

Hardness.

No determinations of hardness were made and the figures given in Table II, which express the degree of hardness on Moh's scale, have been taken from the literature (Hodgman, 1945, Alexander & others, 1944b).

Moisture relations.

The moisture content was measured either by a straightforward gravimetric procedure or by the distillation procedure. In the latter method a known weight of dust was placed in a flask to which 100 cc. of water-free xylene were then added. The flask was fitted with a special trap and condenser. The xylene and water vapour from the condenser fell back into the trap where the water collected and was measured while the xylene returned to the flask (Dean & Starke, 1920, or modernised as A.S.T.M. D95-46). The distillation procedure has the advantage that it only estimates the free water present and it requires less time than the gravimetric procedure but a larger sample is necessary and it is not so accurate for low moisture contents. The results obtained by the two methods will be found in Table III which includes values for dusts in equilibrium with various atmospheric humidities.

TABLE II.
The physical properties of certain non-toxic dusts.

Powder	Specific gravity 25/25°C.	Bulk density cc./g.		Particle size range μ	Specific surface sq.c.m./g.	Hardness Moh's scale
		Large scale	Small scale			
Almicide	3.22	5.1	3.6	<2	29,460	9 approx.
Stockalite	2.64	4.6	2.9	1-2(+), 10(-)	23,210	2-2.5
Devolite	2.63	2.2	1.2	<10(+), 40(-)	8,130	—
Gypsum	2.37	1.4	0.8	<40(+)	5,500	1.6-2
Fuller's Earth	2.64	1.6	1.1	<80(+)	4,640	—
Slate Dust 99/300 Tyler	2.84	1.8	1.2	<80(+)	7,590	3 approx.
Talc	2.78	2.1	1.3	<40(+)	6,280	1-1.5
Neosyl	2.20	7.8	4.9	<1	52,790	7
Sil-o-cel... ..	2.00	11.0	5.4	<10(+)	42,710	—
Calcium carbonate (ppt.)... ..	2.65	1.2	0.8	<10(+), 20(-)	600	—
Carborundum (finest)	3.20	—	0.8	1 to 15	—	9-10
Carborundum (coarsest)	3.20	—	0.6	50 to 200	—	9-10
Lamp black	1.74	—	8.0	1 approx.	—	Indeterminate
Acetylene black	1.95	—	2.8	1 approx.	—	Indeterminate
Carbon black	1.90	—	11.7	1 approx.	—	Indeterminate
Glass (flaky)	—	—	—	2-20(+), 50(-)	—	5 approx.
Glass (round)	—	—	—	2-20(+), 50(-)	—	5 approx.

In the particle size range columns, (+) indicates the size or limit of the majority of the particles; (-) indicates a few particles up to the dimensions to which it is appended also occur.

TABLE III.

The moisture content of certain non-toxic dusts.

Powder	Moisture Content % wt.						
	As received		In equilibrium with R.H. %				
			100	76	60	32	
	By weight	By distillation	By weight				
Almicide	4.7	—	46.0	11.9	6.3	4.1	
Stockalite	1.0	1.0	5.4	0.9	0.8	—	
Devolite	0.5	—	2.6	0.7	—	—	
Gypsum	18.3	18.5	16.0	5.2	—	5.5	
Fuller's earth	9.7	10.9	17.0	10.1	9.0	6.2	
Slate Dust	0.3	0.3	0.5	0.2	—	—	
Talc	0.1	< 0.3	0.4	0.1	—	—	
Neosyl	12.4	13.7	25.0	17.6	13.8	5.9	
Sil-o-cel... ..	7.6	7.5	10.0	5.6	—	1.9	
Calcium carbonate	0.1	< 0.3	0.2	0.05	—	—	
Carborundum (all grades)	—	—	—	—	0.2	—	
Carbon black	—	—	—	—	0.5	—	
Lamp black	—	—	—	—	3.4	—	
Acetylene black	—	—	—	—	7.9	—	

Before the estimations the dusts were stored in thin layers at the humidity indicated for at least two weeks.

Culture and Biology of the Test Insects in relation to the Action of Insecticides.

Large numbers of insects are usually required for experiments with insecticides and it is advantageous to choose species which can be bred easily at all seasons of the year. For this reason, beetles infesting stored products were chosen, although it might be argued that results obtained in this way would not be applicable to insects attacking crops in the open air which is where dusts are mainly employed. However, the application of data obtained on one insect to another is always open to objection and the advantage gained by using one field crop pest, and having data applicable to that insect without modification, is so slight for the present purpose of studying the mode of action of dusts that it seemed to be far offset by the greater advantage of having several easily reared insects available in large numbers. Furthermore, plant pests are much more difficult to rear with constant resistance.

Four species of beetles have been used for the experimental work. They were selected from commonly occurring insects which were known to be easy to breed and because they showed certain differences which seemed likely to be of consequence in relation to insecticidal dust lodgement and action.

Methods of culturing.

All the cultures were kept in a constant temperature room maintained at 25°C. and 60 per cent. relative humidity in 2-lb. jam-jars closed with clip-on metal lids (pierced with 1-in. holes covered with wire gauze) or simply with pieces of muslin held on by rubber bands when the insects showed no tendency to cut their way out. The jars were kept standing in shallow trays of lubricating oil to trap escaping insects and to check the spread of mites.

Of the four beetles, *Tribolium castaneum* (Herbst) was fed on national flour, *Calandra granaria* (L.) and *Rhizopertha dominica* (F.) on whole grain and *Ptinus tectus* Boield. on a mixture consisting of 75 per cent. national flour, 20 per cent. white soluble casein and 5 per cent. dried yeast (Ewer & Ewer 1942). Alternatively fish

meal may be used for *Plinus*. With the exception of the casein and yeast, all the foodstuffs were sterilised in 12-lb. lots by autoclaving at 120°C. for 30 minutes. *Tribolium*, *Plinus* and *Rhizopertha* were given crumpled paper, above the layer of food, to walk upon and adult *Plinus* a tube of cotton wool saturated with water also. This was necessary in the case of *Plinus* to secure maximum oviposition (Ewer & Ewer, 1942).

In all cases the procedure followed in handling the beetles was essentially the same. The requisite number of 2-lb. jam-jars were one-quarter filled with the food and about 200 beetles (or 400 in the case of *Tribolium*) were added. They fed and oviposited in the food for 14 days and at the end of this period they were separated from it with a suitable sieve, or by suction, and the larvae were allowed to continue their development. The date when adults appeared was noted and the cultures were sieved. Emergence was then allowed to take place over 14 days. At the end of this period the adults which had already emerged were sieved off or removed by shaking several times from the papers placed in the culture jars (this procedure caused less injury to *Rhizopertha*) and transferred to fresh food stuff. The remainders of the cultures were usually kept for a further period to gather the beetles that had not yet emerged. *Rhizopertha* needed more careful handling than the other species since it tended to be injured by sieving and was also inclined to gnaw off the appendages of its fellows if kept in overcrowded conditions. The beetles were used for testing when they were 14–28 days old.

Biology in relation to the action of insecticides.

For general information concerning the biology of the four beetles used in the present experimental work, reference should be made to the original papers. *Tribolium* has been described by Good (1936), *Calandra* by Andersen (1934) and Back & Cotton (1926), *Rhizopertha* by Potter (1935) and Birch (1945) and *Plinus* by Hinton (1941), Hickin (1942), Howe (1943) and Gumm & Knight (1945). It is therefore unnecessary to consider the biology of these insects in any general way, but, since the properties of a dust affect its insecticidal action, it would be expected that correlations might likewise be found between the structure and behaviour of insects and their response to insecticides. The four species concerned will be considered from this viewpoint and an attempt will be made to show how the structure of the beetles is likely to influence the retention and subsequent action of the insecticidal dusts.

The general shape of an insect will influence the lodgement of dust on its integument, and, where the outline is irregular and presents areas not freely brushed against the environment as the insect moves, the lodged material will tend to remain in position. On this ground alone the smoothly cylindrical *Calandra* would be expected to retain less dust than the more irregular *Rhizopertha*.

A smooth beetle, such as *Tribolium*, will retain less dust than other beetles which are more hairy. In the same way the hairy regions will retain the most dust, but hairiness on the other hand may tend to inhibit insecticidal action if the hairs keep the insecticide from reaching a site of effective action or absorption. Certainly the felt-like covering of hairs on *Plinus* is not easily penetrated by dust particles.

Dust tends to lodge at body and limb joints where the intersegmental membranes or articulating surfaces are often much thinner than the general integument. The access of insecticide to such surfaces is therefore likely to be of some importance. The cervicum and the prothoracic-mesothoracic junction are the main body joints. Except in the case of the cervical joint in *Calandra*, all these junctions have combs of hairs which tend to prevent particles reaching the articulating surfaces and which brush away any which do get there. The cervical articulation of *Calandra* is protected by the thin membranous margin of the prothorax which forms a very close junction with the smooth ball-like posterior surface of the head. The comb of hairs occurring in all beetles except *Calandra* is, however, unlikely to afford protection for the thin

intersegmental membrane which is exposed by these beetles at either junction during more extreme movements. Wigglesworth (1947) has shown that dust does gain access to these surfaces to a limited extent and also to the limb joints.

The dorsal surface of a beetle, protected by a hard elytra, is very often thin and this is true of the four test species. Since it is also usual for the openings of the abdominal and thoracic spiracles to be covered by the margins of the elytra it is important to decide how readily dust passes beneath them. In *Calandra* the elytra are partly fused together and to the sides of the abdomen and dust cannot penetrate beneath them except from the posterior end. In the other three species the elytra can be moved and access of dust is facilitated. In the case of an abrasive dust the damage usually occurs along the lateral margins of the abdomen and elytra but except for the last few segments little occurs on the dorsal surface and it seems doubtful if dust usually reaches this area. Dust was seldom found in the spiracles and then usually only to the extent of one or two particles in a single spiracle.

Dust particles cling to the mouth-parts of insects and are known to be eaten by them although *Calandra* does not do so unless some food material is mixed with the dust. Dusts also cling to the antennae and they and the palps often carry chemoreceptors which may be more permeable to insecticides than the general cuticle. In some cases the tips of the antennae are very hairy (*Calandra*) or the distal part of the terminal joint of the palps may be very thin walled (*Tribolium*) and these regions may then be important sites of insecticide entry.

Well developed compound eyes are present in all species and the lenses are frequently much scratched when the beetles are exposed to abrasive dusts. It seems possible that the eyes will differ from the rest of the cuticle in their permeability to insecticides.

When the four test beetles were immersed in a variety of dusts no very unexpected behaviour was observed. On the relatively smooth *Tribolium* the quantity of dust which adhered to the general surface was very slight but there was more in the region of the mouth and the pro-mesothoracic junction. *Calandra* is more hairy and more dust clings to it and, as would be expected, very much more clings to *Rhizopertha* and *Plinus*. In certain dusts which readily form aggregates, the hairy insects become covered over almost entirely with very thick coats of the semi-compacted powders which continue to adhere as the insect walks around.

With all the beetles, it was noticeable that certain dusts appeared to be much more adherent than others. Very little Alnicide and Calcium-carbonate adhered to them, slate and talc were much more adherent, while on *Rhizopertha*, Neosyl (silica) and Stockalite (kaolin) formed heavy compacted coats.

Assessment of Results.

The beetles were examined each day during their exposure to the dusts and any which showed the slightest movement after careful examination were counted as being alive. *Calandra* and *Plinus* had a marked tendency to remain motionless and so appeared to be dead but this could usually be overcome by a touch or by breathing gently upon them. Living beetles usually folded their legs against their bodies, whereas in dead insects they were more or less stretched out. The position of the legs was thus a useful guide to their true condition.

In the course of the six days for which the tests were usually continued, there were normally no deaths among the control beetles except *Calandra*. When the control deaths exceed 5 per cent., they are either indicated or corrected for by Abbott's formula (1925).

The results obtained in the biological tests are shown in tabular form or graphically as sigmoid mortality/time curves, except in certain cases where the latter have been converted to provisional regression lines (Bliss, 1935).

BIOLOGICAL TESTING METHODS.

Insects can be brought into contact with a dust under a variety of conditions. For example, they can be immersed in an excess of the dust for the duration of the experiment or separated from it after a brief exposure. Alternatively, they may be exposed to a settling dust cloud or to a substratum on which the dust has already been allowed to deposit.

The first condition of exposure listed above is the simplest, since with uninterrupted contact all factors influencing the accumulation and adherence of the dust are eliminated and the method therefore permits fairly straightforward determinations of toxicity to be made. If, on the other hand, the insects are separated from the bulk of insecticide after a brief period of exposure by some such process as sieving, or are not in constant contact with an excess of dust, adherence immediately becomes an operative factor. The situation is further complicated if the dust is applied as a cloud since the behaviour of the particles in the cloud has then also to be taken into account.

Continued contact test.

The continued contact tests were made in specimen tubes 3 in. long and about 1 in. in diameter since these were available in large numbers. The tubes had the advantage that the dusted beetles could not climb out of them and only control tubes of *Calandra*, which can climb glass in the undusted condition, had to be closed with gauze.

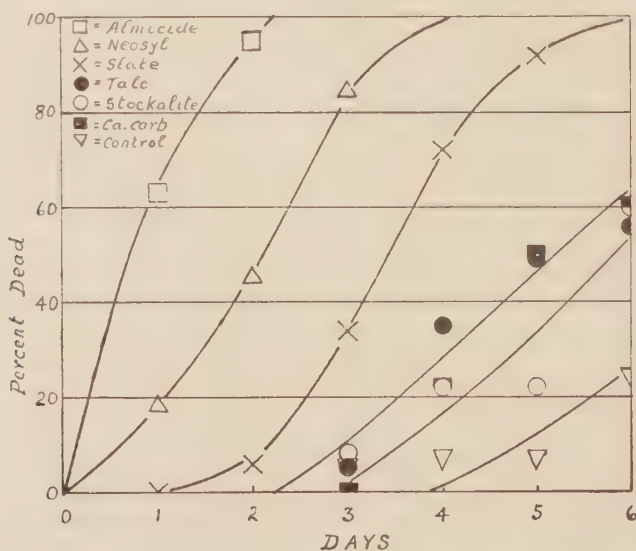


Fig. 1.—The continued contact method with *Calandra* showing differences in the desiccating power of the various dusts. The tests were conducted at, and the dusts conditioned to, 60 per cent. R.H. at 25°C. The beetles were between 14 and 28 days old at the beginning of the test and each point on the graph was determined by averaging the results of three tests each employing 20 beetles.

Unless all the beetles had died before, the tests were continued for six days and a set of three tubes were prepared for each day except when experience suggested that days at the beginning or end of the test could be omitted. It will be noted that the

results for each day were therefore independent of those on the previous or subsequent days when using this method. Each tube contained about 20 beetles and about $\frac{1}{4}$ in. of dust. On the day of examination they were separated from the dust by sieving and inspected carefully for any signs of movement. A beetle capable of the slightest twitching movement was counted as alive. Since the beetles feign death it was necessary to wait an adequate time before deciding that a creature was lifeless.

At a later stage in the investigation, a method using less dust and fewer insects, that did not involve sieving them from the dust before counting, was employed. In this method a little of the dust was placed in a 2-in. diameter petri dish together with the beetles. Three or four dishes were prepared for each test and the beetles were re-examined each day.

The procedures just described were carried out at 25 C. and at relative humidities of 0 per cent., 60 per cent. or 100 per cent. to which the dusts had been conditioned for a prolonged period beforehand.

Discontinued contact test.

In the discontinued contact test, the insects were exposed to the dust for a fixed period and then sieved free from excess by means of an improvised mechanical sieve operated in a constant manner. It was expected that the procedure would leave a fairly constant quantity of dust adhering to the insects and permit an investigation to be made into the relationship between the properties of the dusts and the amount adhering and the influence of the latter on the observed kill. After the insects had been removed from the sieve they were transferred to filter papers and covered over with petri dishes. The tests were made in triplicate and the insects were recounted for six days if they had not succumbed before.

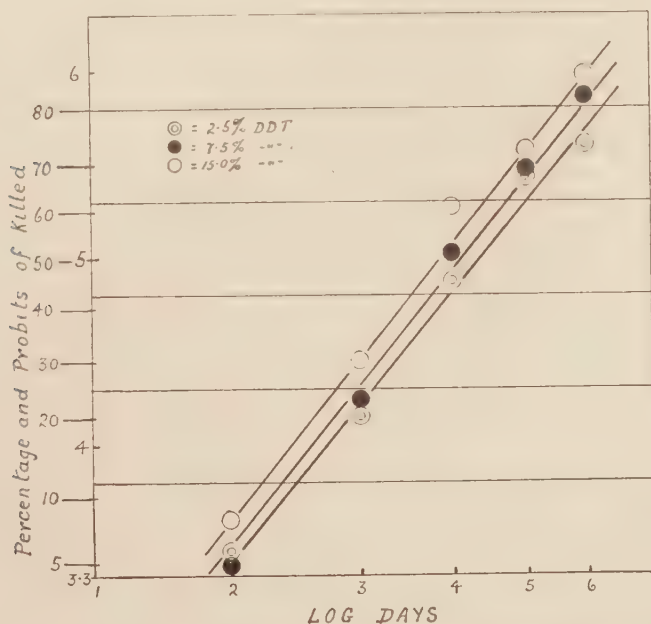


Fig. 2.—The continued contact method using *Calandra* did not provide a sensitive measure of the percentage of DDT in the dusts. Conditions as in fig. 1, except that the relative humidity was 100 per cent.

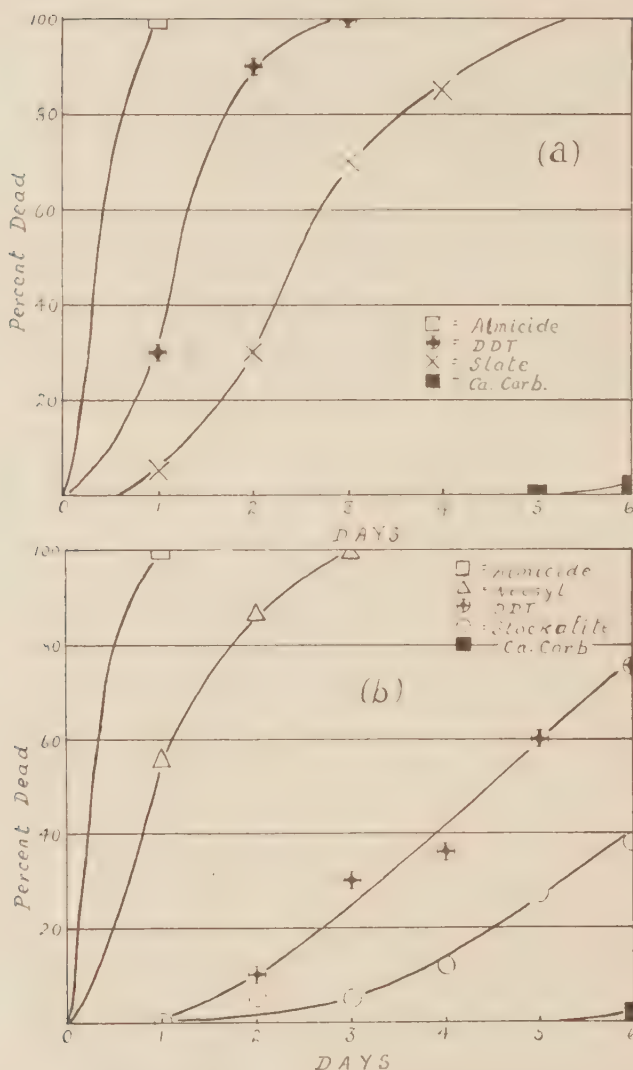


Fig. 3.—(a) *Tribolium* and (b) *Rhizopertha* may be killed more rapidly by the desiccating action of the dusts than by pure DDT when tested at 25°C. and 60 per cent, R.H. The beetles were under 28 days old and each point on the graphs was determined with three lots of 20 beetles.

Assessment of the Quantity of Dust adhering to Insects.

Two methods were used to determine the quantities of dusts adhering: the first involved weighing the insects before and after dusting, while the second was a colorimetric method.

Weighing method.

When beetles were exposed to dusts and the excess was removed by sieving, the quantity which remained adhering was never very great and in the case of smooth-coated beetles could hardly be weighed. It was therefore necessary to take precautions

to eliminate other weight changes which the beetles might undergo. The beetles were removed from their food about 18-24 hours before the experiment was commenced as this allowed time for particles to be detached from the integument and for excrement to be voided. During the conditioning period the beetles were kept in well-ventilated dishes and given paper to walk on so that they were not over-crowded. At the commencement of the test exactly 50 or 100 insects were counted out, weighed, exposed to the dust for five minutes, sieved and reweighed.

The weighing method had the advantage that it could be used with the untreated dusts and the insects actually concerned, so that a direct measurement of the quantity

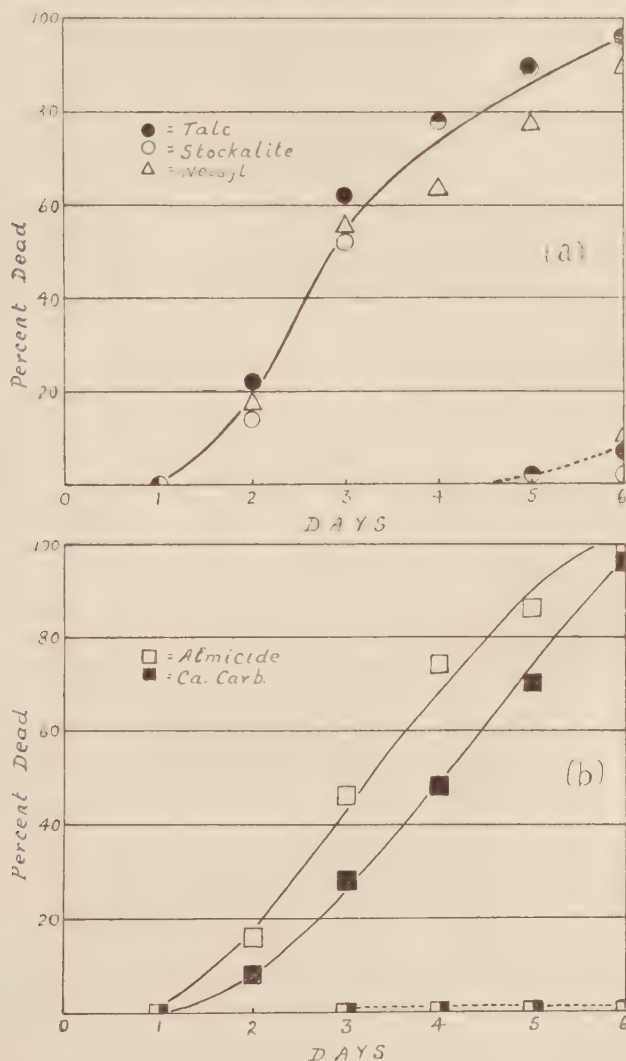


Fig. 4.—Under saturated atmospheric conditions the non-toxic dusts were without effect on (a) *Calandra* or (b) *Tribolium*, but when there was DDT in the dusts it continued to exert its action. Conditions as in fig. 1. The dusts containing 5 per cent. w/w DDT are represented by unbroken lines, the same dusts without DDT by broken lines.

of material adhering was obtained. It could not be used to determine the persistence of the dust on the insects since other weight changes supervened, mainly by loss of water.

Colorimetric method.

For the colorimetric method the beetles may be handled previously to the test as described in the weighing method or may be removed directly from the culture. The dust concerned must be dyed and this can be done most readily by treating a weighed quantity with a solution of a known concentration of the dye in a volatile

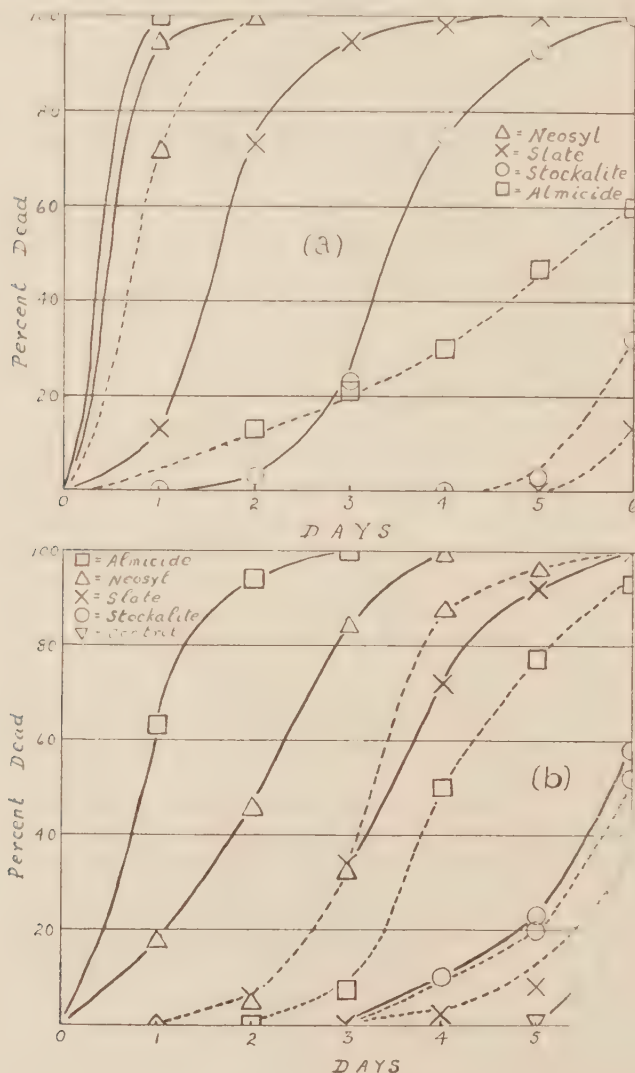


Fig. 5.—With the smoother beetles (a) *Tribolium* and (b) *Calandra* the discontinued contact method (represented by broken lines) of comparing the non-toxic dusts gave lower kills than the continued contact method (unbroken lines)—also, the relative order of effectiveness was sometimes different. Conditions as in fig. 1. The duration of exposure in the discontinued tests was 1 hour.

solvent. Sudan III in benzene was found to be most satisfactory for this purpose. The benzene was removed by heating in a water bath and a further short period in an oven at 105 C. The insects were exposed to the dyed dust for five minutes, sieved, and then ten were dropped into 2-4 cc. of 50:50 alcohol and benzene. A set of 1 cc. standards was prepared by extracting a known weight of the particular dyed dust with the same solvent mixture and diluting (David 1946a). The solvent mixture was more satisfactory for extracting the dye than benzene alone, especially if the dusts were slightly moist.

The method must be slightly modified for *Tribolium* otherwise the colorimetric estimation is interfered with by the yellow discoloration caused by the secretion of the stink glands. This difficulty can be overcome by chilling the insects and the solvent separately in a refrigerator at 0 C. and decanting the extracting fluid as soon as the dye is dissolved.

The colorimetric method has the disadvantage that it involves dyeing the dust under examination and thereby probably modifying its properties either by the direct addition of the dye or in the course of the handling during application. On the other hand the method can be used to follow the persistence of the dust on the insect—though if any dust is removed by the cleaning operations and swallowed, or if the dye penetrates the cuticle it will not be estimated. With the particular test insects concerned, it was shown that a certain amount of dust was eaten but there was no evidence that any of the dye was lost by absorption through the cuticle.

Comparison of the results obtained by weighing and colorimetric methods of measuring adherence.

An experiment was made to compare the results obtained by the weighing and colorimetric methods of estimating the quantity of dust adhering and to test the repeatability of each method.

Stocks of *Calandra* and *Rhizopertha* were separated from their foodstuffs and starved overnight. Several lots of 50 beetles were counted, then weighed, dusted, separated from excess dust, re-weighed and finally extracted in the same tube with the alcohol/benzene solvent. For these tests dyed Almicide, Stockalite and talc were used. From the results given in Table IV, it can be seen that there was a general agreement between the two methods but that the results by the colorimetric technique were more consistent.

TABLE IV.
Results of the gravimetric and colorimetric methods.

Dust	Test No.	Quantity adhering mg./g.							
		<i>Calandra</i>				<i>Rhizopertha</i>			
		By Weight		By Colour		By Weight		By Colour	
		Repeat	Average	Repeat	Average	Repeat	Average	Repeat	Average
Almicide ...	1	4.6		6.1		7.8		10.4	
	2	4.8		6.4		13.4		9.7	
	3	3.7	4.4	5.9	6.1	9.8	10.3	9.9	10.0
Stockalite	1	17.2		20.9		36.2		33.8	
	2	3.8		18.5		37.4		43.4	
	3	11.3	10.8	14.9	18.1	31.2	34.9	33.8	37.0
Talc ...	1	15.2		10.5		17.1		17.0	
	2	12.0		10.5		33.8		17.0	
	3	8.1	11.8	10.4	10.5	14.5	21.8	19.5	17.5

Experiments relating to the Testing Methods.

In the following sections details are given of some experiments which were especially designed to investigate various aspects of the biological methods and the methods used for assessing the quantity of dust adhering to the insects.

Sensitivity of the continued contact method.

Although the method shows up clearly the differences in the desiccating power of various dusts (fig. 1), it does not, with the test beetles chosen, provide a sensitive method of comparing the DDT content of dusts; indeed it is remarkably insensitive (fig. 2). This insensitivity is probably attributable to the fact that the beetles are very resistant to DDT and only react slowly to relatively high concentrations. Indeed, unless the air is almost 100 per cent. saturated with moisture the beetles may be killed more quickly by the desiccating action of the carrier dusts (even when this is not very pronounced) than by pure DDT (fig. 3a and b). In order to determine the response of the beetles to DDT alone it is therefore necessary to conduct the tests at 100 per cent. R.H. Under these conditions very few beetles die in the carrier dusts and the effect of the DDT can be distinguished (fig. 4a and b).

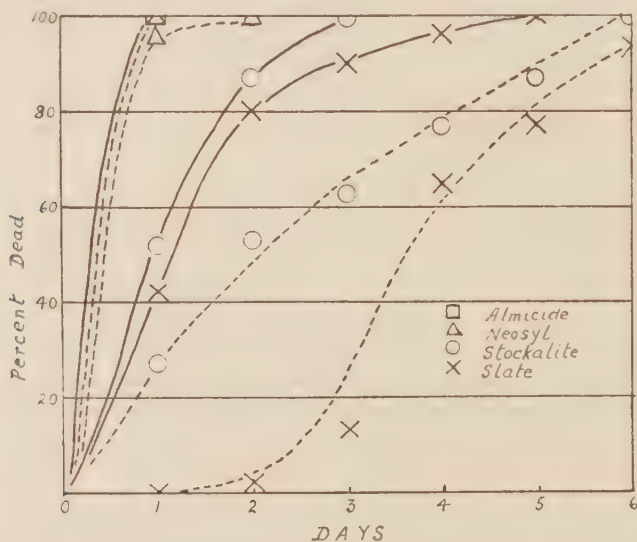


Fig. 6.—With *Ptinus*, which is rough coated and held a large quantity of dust, the order of effectiveness of non-toxic abrasive dusts when compared by the continued and discontinued contact methods was the same. Conditions as in fig. 1. The duration of exposure in the discontinued method was one hour. The results of the discontinued tests are shown by broken lines, of continued by unbroken lines.

Sensitivity of the discontinued contact method.

The discontinued contact method, like the continued contact method, also shows the differences in the desiccating properties of the various dusts and not only is the effect produced less but the dusts sometimes assume a different order of effectiveness. This is well seen in the case of *Tribolium* and *Calandra* where the reversal of order is marked (fig. 5a and b) while no such effect can be seen with *Ptinus* (fig. 6). It seems probable that this reversal of the order of effectiveness occurs because of the differences in the adherence of the dusts which tend to be especially evident on the smoother beetles. Certainly Almicide is less adherent than Neosyl and although always the more lethal in the continued contact tests it becomes inferior in the discontinued contact tests on the smoother beetles. This does not happen with *Ptinus*, however,

probably because, as is known to be the case, the quantity adhering is large even in the case of Almicide and is sufficient for it to exert an effect almost equal to that obtained with continued contact.

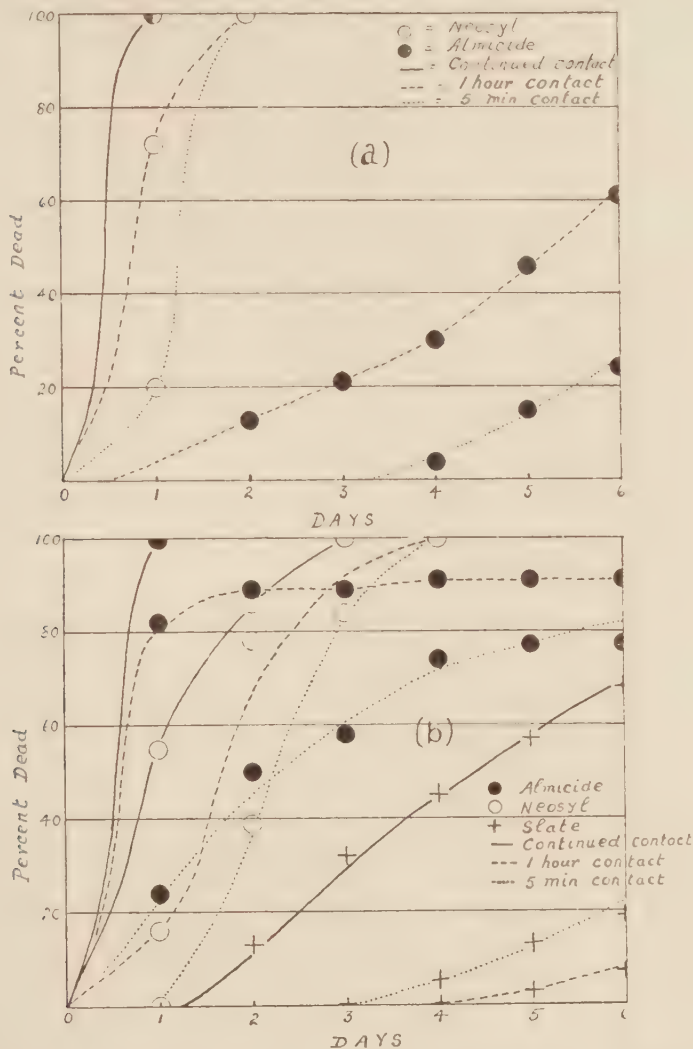


Fig. 7.—Non-toxic dusts killing (a) *Tribolium* and (b) *Rhizopertha* by desiccation were more effective in the discontinued contact test when the contact period was increased from 5 to 60 mins. and the kill by the continued contact method was greater than either. Conditions as in fig. 1. Unbroken lines=continued exposure, dashes=60 mins. exposure, dots=5 mins. exposure.

Influence of the duration of the contact period on the kill.

For the discontinued contact method, the beetles were exposed to the dust for a limited period only and then sieved free of the excess. The kills obtained were thereafter recorded on each day for six days. Experiments were made to show the effects that alterations in the exposure period had on the number of insects killed

in the case of non-toxic dusts acting by abrasion (fig. 7a and b) and DDT dusts at 100 per cent. R.H. where only the DDT exerted a killing action (figs. 8 and 9).

From the results given in fig. 7a and b, it may be concluded that with the non-toxic dusts an increase in the contact period leads to an increase in the kill, the effect being especially evident when a smooth-coated species is used and the dust is not very adherent, *Tribolium* and Almicide (fig. 7a), but still evident with *Rhizopertha* and the more adherent Neosyl (fig. 7b).

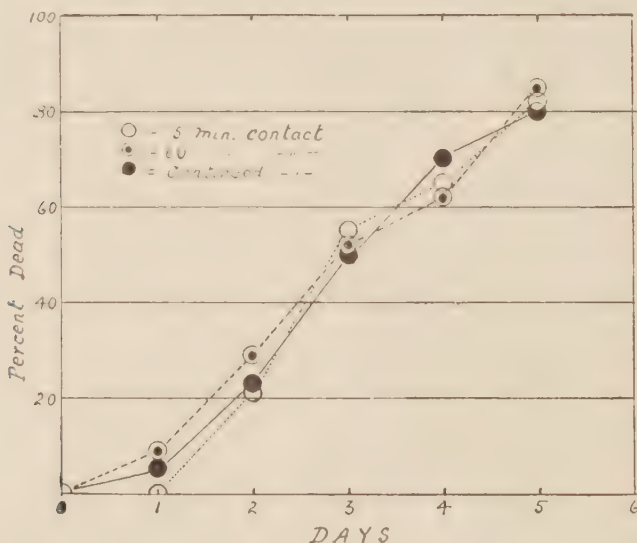


Fig. 8.—Prolonging the exposure of *Calandra* to a dust of 7.5 per cent. DDT on Neosyl from 5 to 60 mins. by the discontinued contact procedure did not increase the kill which was in any case not significantly different from that obtained by the continued contact technique. Conditions 25°C., 100 per cent. R.H. Unbroken lines=continued contact, dashes=60 mins. exposure, dots=5 mins. exposure.

In the case of DDT dusts tested at saturated humidity *Rhizopertha* and *Ptinus* were too resistant to DDT to give satisfactory results but with *Calandra* and 7.5 per cent. DDT on Neosyl there was no consistent difference in the kills obtained with 5-min., 60-min. and continued exposures (fig. 8).

Generally speaking, however, the continued contact method tends to give higher kills than the discontinued contact method. This was found to be the case with *Calandra* exposed to 5 per cent. DDT in kaolin and to a much more marked degree with *Tribolium* to which very little dust adheres (fig. 9).

Sieving and dust adherence.

The mechanical sieve used in the discontinued exposure technique for separating the beetles from the excess of dust to which they had been exposed was operated by tapping. It seemed possible that by increasing the number of taps administered the quantity of dust adhering to the insects could be reduced and that this would provide a method of controlling the dose and so studying its relationship with the resultant kill. In fact it was found that a gradation in the dose could not be produced by this method and that a certain number of taps was necessary to pass the excess of dust through the sieve. Further tapping had very little effect on the quantity of dust adhering to the insect and consequently the kill showed no tendency to fall off as the number of taps was increased. If, instead of sieving, the insects were dropped

about six times from about three ins. on to filter paper to dislodge the dust, the quantity found adhering was the same as that left after sieving. It appears therefore rather as if this amount represents firmly adherent material which is not easily dislodged by mechanical action. It is, however, known to be lost slowly as the insect crawls and kicks during the period following exposure. The results obtained are given in Table V.

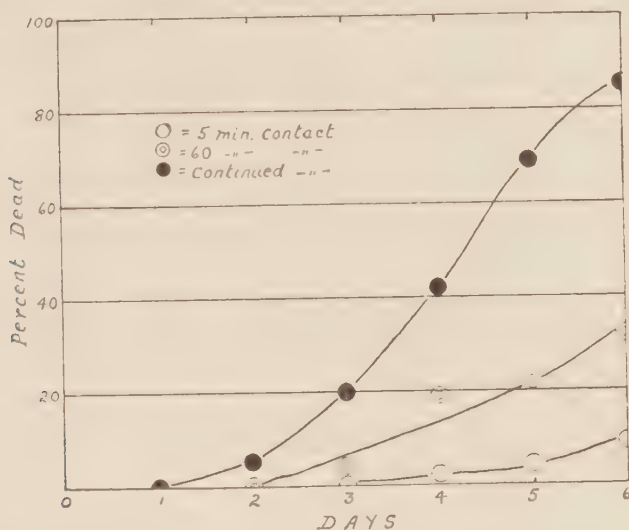


Fig. 9.—Prolonging the exposure of *Tribolium* to a dust of 5 per cent. DDT on kaolin from 5 to 60 mins. led to an increase in the kill but in this case, where very little dust tended to adhere, the kill did not nearly reach that obtained under continued contact conditions.

TABLE V.

Effect of mechanical vibration on the quantity of dust (kaolin containing 10 per cent. DDT) that remained adhering to *R. dominica* determined colorimetrically.

No. of taps etc. given	Quantity found adhering mg./g. insects	Percentage kill on day		
		4	5	6
10	23.8	28	31	33
20	17.5	33	41	43
50	17.5	29	33	35
Dropped	17.5	28	29	43
Control	0	0	0	0

After exposure to dusts the insects were kept at 25°C. and 100 per cent. R.H. The figures for kills were each based on four determinations using 20 insects for each.

Effect, on the adherence and toxic action, of the substratum on which the insects were placed after being exposed to dusts.

In the discontinuous contact method of testing dusts, the insects which have been separated from the dust after the prescribed period must be kept for several days in order to follow the effects of the insecticide and a suitable enclosure was necessary. A petri dish inverted on a slightly larger glass plate, on a filter paper backed by the

glass plate or on a filter paper with some flour and wheat grains were all tested. The quantity of insecticide that remained adhering to the insects over a period of several hours was most when the insects were on plain glass and least on filter paper with food. Glass did not provide a satisfactory foothold for insects and any that were slightly affected by insecticide could not right themselves after falling on to their backs. This happened on filter papers also but only at a more advanced stage of poisoning. There seemed to be little advantage in supplying food, and filter papers were used in the standard procedure. The progressive loss of Stockalite (kaolin) under the three recovery conditions was determined and it was not surprising to find that dislodgement was slowest in the smoothest environment.

TABLE VI.

Effect of different substrata on the loss of Stockalite adhering to *R. dominica*.

Substratum	Time after removal from dust Hour			
	0	1	5	18
Glass	41.0	25.0	13.0	9.0
Filter Paper	41.0	15.5	4.0	3.5
Filter Paper and Food	41.0	5.0	1.0	1.0

The figures in the body of the Table show the weight of dust adhering as mg. of dust per g. of beetles.

When the adherence of a dust was followed over a longer period, it was found that very little more was lost after the toxic action became apparent since the beetles lost their ability to walk and fell over on their backs. This happened on about the second day with *Rhizopertha* in 10 per cent. DDT dust and after this time until the end of the experiment on the sixth day the quantity of dust adhering remained almost constant. This quantity was less when the beetles were kept on filter paper than on glass and the difference was correlated with a slightly lower percentage kill in the former case. Since the test was conducted at 60 per cent. R.H. it was uncertain whether the slightly greater percentage of insects killed when more dust adhered was due to the DDT or to the increased desiccating action. Probably both factors could have contributed. Essentially similar results were obtained with kaolin and slate, to which 10 per cent. wt. of DDT had been added (Table VII); it will be noted that the dusts are lost more quickly on the filter paper, and the kills are slightly lower.

TABLE VII.

Influence of the substratum on which *Rhizopertha* are placed after treating with DDT dusts, on the persistence of the dust on the insect and the observed kill.

Dust plus 10% DDT	Recovery conditions	Quantity of dust adhering in mg./g. of beetles after :			Percentage mortality after days :			
		0 hr.	1 hr.	24 hr.	3	4	5	6
Kaolin	On plain glass	49.8	28.6	19.9	17	24	33	43
	On filter paper	49.8	14.9	5.0	14	22	27	35
Slate	On plain glass	24.9	12.5	14.9	18	22	31	47
	On filter paper	24.9	10.0	9.3	16	19	26	34

The Properties of Non-Toxic Dusts in relation to Insecticidal Action.

A description has already been given of the physical properties of certain non-toxic dusts and it has been pointed out that these dusts may be effective insecticides if they are sufficiently abrasive to damage the insect's cuticle and so promote loss of moisture. It is the purpose of the present section to describe investigations into the relationship between certain properties of these dusts and the insecticidal effect which they produce.

Particle size and adherence.

In many published papers evidence will be found that, in a variety of circumstances, fine powders adhere more readily to surfaces than coarse powders (Alexander & others, 1944b; Fitzgibbon, 1943; Smith & Goodhue, 1942; McGregor, 1934; Wilcoxon and McCallan, 1931; Streeter & Rankin, 1930). Below about 2μ diameter, however, the powders do not become more adherent (Heuberger, 1942), perhaps owing to the marked tendency of such powders to form aggregates which then constitute the operative particles.

By the weighing method of measuring adherence (p. 16) it can be shown that a greater quantity of fine than coarse Carborundum adheres to insects. This observation is true for both smooth and hairy insects as may be seen for *Calandra* and *Ptinus* (Table VIII). The particle size distribution of the Carborundum powders is given in fig. 11.

TABLE VIII.

Test Insect	Weight adhering to 50 beetles mg.							
	Grade of Carborundum Powder							
	120	180	240	320	400	500	600	700
<i>Calandra granaria</i>	0.0	0.0	0.8	0.8	3.1	3.9	4.1	5.2
<i>Ptinus tectus</i> ...	0.0	0.0	0.4	1.6	5.5	8.8	12.6	13.7

While experiments with one powder showed quite clearly that the finer grades were more adherent than the coarser grades, the same conclusion did not apply when different powders were compared. This was not surprising since the powders

TABLE VIII(a).

Dust	Particle size range (Microns)	Specific surface sq. cm./gm.	Quantity of dust adhering $\mu\text{g.}/10$ beetles			
			<i>Calandra</i>	<i>Rhizopertha</i>		<i>Ptinus</i>
				(a)	(b)	
Silica ...	<1	52,790	460	710	710	1,860
Sil-o-cel ...	<10(+)	42,710	310	500	510	2,380
Almicide ...	<2	29,460	130	170	105	600
Kaolin ...	1-2(+), 10(-)	23,210	710	840	810	2,680
Slate ...	<80(+)	4,640	650	840	940	2,840
Calcium carbonate ...	<10(+), 20(-)	600	430	500	520	1,320

When different dusts were compared the finest, as determined by microscopic examination after dispersal in a wetting agent and by specific surface measurement, was not more adherent than the coarsest. The determination of adherence was made by weighing 50 or 100 insects before and after exposing to an excess of the dust for five minutes. Each determination was made in triplicate and the average taken. The experiment with *Rhizopertha* was confirmed. (+) indicates that the figure preceding it refers to the majority of the particles, (-) that a few up to this size also occur.

differed in other respects besides particle size and the state of aggregation in every case, under the conditions of the biological tests, was probably not accurately represented by the determinations of specific surface area and the general particle size range. Besides the properties associated with the powders, however, the structure of the cuticles of the insects to which they adhered must also be considered. It seems probable that only small particles would cling to smooth-coated beetles but that much larger particles would lodge among the hairs of *Rhizopertha* or *Ptinus*. This question needs detailed investigation for both plain carriers and toxic dust mixtures, but meanwhile it can be seen (Table VIIIa) that there was little correlation between the observations made on general particle size and the specific surface of the dusts and their tendency to adhere to insects as determined on the plain dusts by

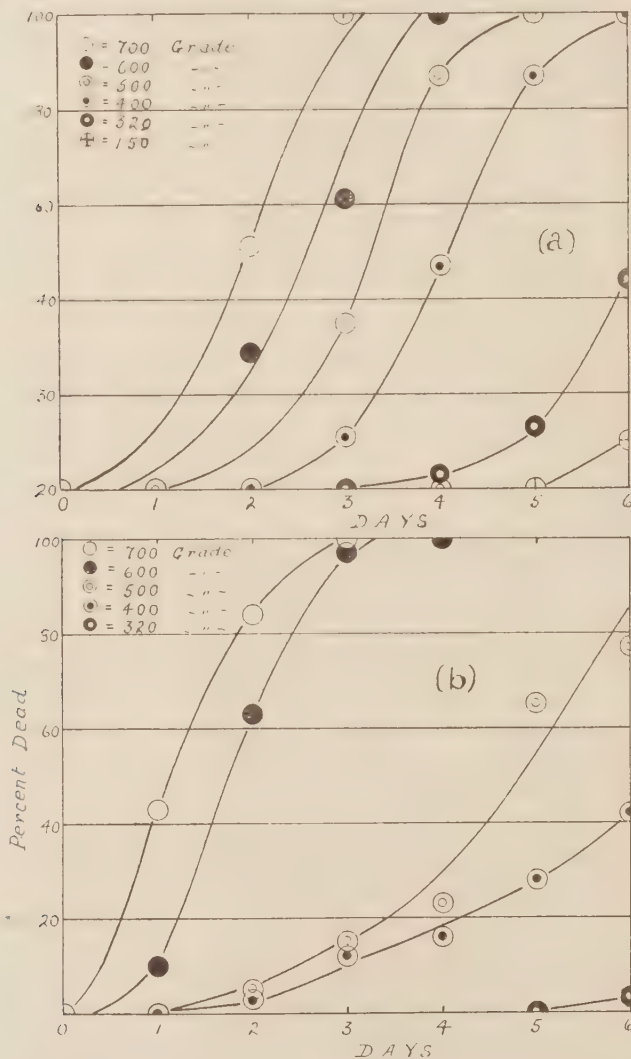


Fig. 10.—(a) *Calandrya* was killed more rapidly by the finer grades of Carborundum than the coarser when exposed to them by the continued contact method at 25°C. and 60 per cent. R.H. The same is true for (b) *Tribolium* in Aloxite grades.

the weighing method. (Colorimetric measurements made with dyed dusts did not agree with the weighing method on plain dusts. It is probable that the state of aggregation of a dust was altered during dyeing and that this affected the adherence since the results by the two methods agreed when the same dyed dusts were used for both determinations (p. 19).)

Particle size and abrasion.

It has been shown by Chiu (1939) that particles of silica under $5\ \mu$ are more lethal to insects than particles more than $15\ \mu$ in diameter. It has also been shown that particles of smaller diameter adhere well to insects whereas above about $15\ \mu$ adherence is low (Alexander & others, 1944b) and it might be supposed that it was this failure to adhere on the part of the larger particles that accounted for their ineffectiveness. This, however, is not the case since beetles left in continuous contacts with an excess of powders of varying particle size, so that they were always surrounded by dust, are much less affected by coarse powders than by fine (Alexander & others, 1944b). This observation is clearly confirmed in experiments with Carborundum and Aloxite (fig. 10a and b), the particle size distributions of which are shown in fig. 11. The same is true of slate and talc fractions prepared by elutriation (fig. 12a and b). In each case there is an increase in the rate of death by desiccation with a reduction in the average particle size.

Wigglesworth (1944, 1945, 1947) has demonstrated that hard abrasive powders exert their lethal action on insects by damaging the cuticle and so promoting water loss. The abrasion is seen in certain areas only, such as the mouthparts, the leg joints and the sides of the abdomen, under the margins of the elytra; in fact, with the exception of the tips of the appendages, where two parts of the body move against each other. It seems probable from these observations that the coarser particles are ineffective as abrasives because they fail to penetrate between the surfaces. If the occurrence of abrasion is taken to indicate penetration to the articulating surfaces it can be shown by staining with ammoniacal silver nitrate (Wigglesworth, 1945) that this is much more pronounced after exposure to the finer powders and that in their absence, an increase in the amount of staining beyond the level found in the control, there is no kill. Likewise it may be shown that more intense staining follows exposure to the finer powders and that the lethal action of the powders was correspondingly greater. The degree of abrasion was assessed on cleared mounted insects by scoring 0-3 according to the intensity of staining at the several places where it occurred. In order to avoid bias, the score was assessed without knowing the treatment that the insects had been given. Only the finer grade increased the abrasion score above the control figure and had a lethal action.

TABLE IX.
Rhizopertha exposed to two grades of Carborundum.

Treatment	Total abrasion score	Percentage kill observed after days					
		1	2	3	4	5	6
Control	37	0	0	0	0	0	0
Carborundum 150-coarse	33	0	0	0	0	0	0
Carborundum 700-fine ...	64	0	0	25	43	53	65

Tested at 25°C. and 60 per cent. R.H. The abrasion was assessed on three insects and the controls were exposed to ammoniacal silver nitrate.

Small particles are eaten more readily than large particles.

It is not difficult to show that insects will eat a great variety of powders of no food value either directly or in the course of cleaning their antennae and wings by

drawing them through the mouthparts (Shafer 1915, Mote & others, 1926, Wilcox, 1926).

Tribolium, *Rhizopertha* and *Plinus* all ate several dyes, carborundum powders and lamp blacks but *Calandra* did not do so unless the material was mixed with a foodstuff such as a flour. All these materials were seen easily in the gut after a simple dissection. It was, of course, much more difficult to see colourless materials and generally speaking the non-toxic dusts had to be dyed before they could be detected in this way. This difficulty may account for the failure of Germar (1936) to find any trace of powdered sand in the digestive system of dusted weevils although in the absence of food the weevils would probably not in fact have eaten the dust.

The maximum size of the particles consumed will ultimately be limited by the size of the mouth opening and Shipitzina (1935) has shown that this factor limits the size of particles consumed by mosquito larvae. A similar effect has been found with the graded Carborundum powders—the finer grades were eaten more readily than the coarser grades and the coarsest grades were not eaten at all (Table X). *Calandra* did not eat Carborundum but the results show that for the other species fine powders were eaten more readily than coarse. See fig. 11 for particle size data on Carborundum powders.

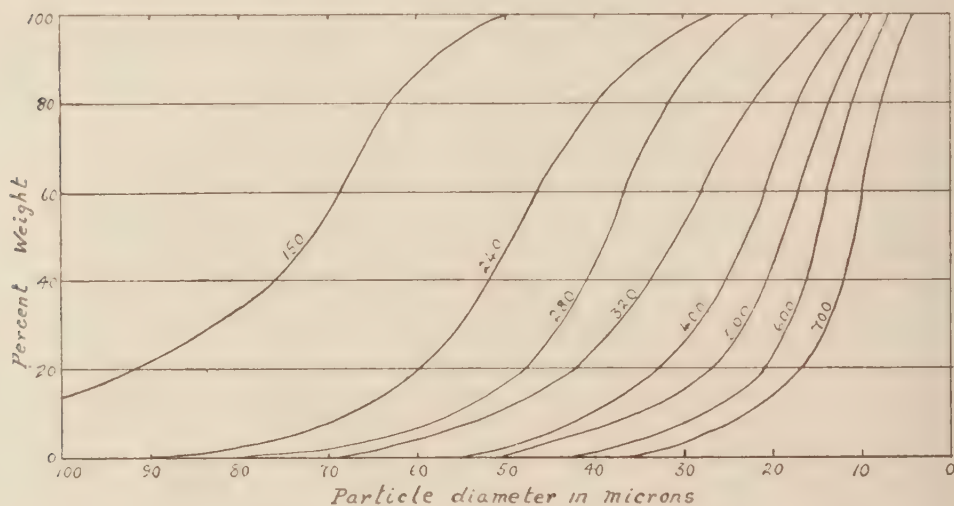


Fig. 11.—Average sedimentation accumulation curves for Carborundum and Aloxite powders showing percentage weight plotted against particle size in microns. The curve for the 150 grade is an approximation. Data kindly supplied by the Carborundum Co.

Small particles enter the spiracles more readily than large particles.

Although it is now recognised that non-toxic dusts produce their effect on insects through abrasion and not because they block the spiracles, as was at one time considered possible, it is still of interest to study the entry of substances into the respiratory system. Detailed investigations into this subject are already in hand. Meanwhile it may be reviewed in relation to the entry of dusts of various particle sizes.

Hockenyos (1933) submerged *Blatta orientalis* in finely powdered magnesium carbonate and also suspended the insect in a dust cloud, but although the spiracles opened to as much as 50μ and the dust particles were all below 10μ in diameter none were found in the tracheae. This conclusion, which may perhaps be accounted for by the difficulty of detecting the dust, is somewhat surprising in view

of the observations of other workers. For example, Hamilton (1937) has shown that cyanide dust under 53μ in diameter was fine enough to enter all the spiracles of *Locusta* and *Schistocerca* and by the relative quantities present it could be deduced that only some were inspiratory. It was not considered that the material contributed to the kill. Webb (1945a) showed that rotenone dust entered the spiracles of *Mclophagus orinus* and that it also increased the rate of action. Both authors concluded that the finer particles entered more readily.

TABLE X.

Powder offered			Hours on powder	Insect			
				<i>Tribolium</i>	<i>Calandra</i>	<i>Rhizopertha</i>	<i>Ptinus</i>
Carborundum 120	5	0	0	0	0
			24	0	0	0	0
			48	0	0	0	0
Carborundum 280	5	0	0	0	0
			24	5	0	4	0
			48	4	0	6	0
Carborundum 400	5	0	0	0	0
			24	6	0	6	4
			48	5	0	5	3
Carborundum 700	5	3	0	1	2
			24	5	0	6	2
			48	3	0	6	4

The figures in the body of the table show the number of beetles out of six examined which had Carborundum in the gut.

Roy & Ghosh (1944), working with the blue bottle fly, *Chrysomya megacephala* (F.), have reported that while normal insects dusted with pyrethrum begin to be affected in a few minutes those with their spiracles blocked did not respond. Alexander & others (1944a), after microscopic examinations, failed to find any of a variety of dusts in the spiracles of *Calandra* but this is perhaps not surprising since they are well protected by the more or less immovable elytra.

Observations have been made on the entry of dust particles into the spiracles of *Calandra*, *Rhizopertha* and *Tribolium*. No particles have ever been detected in the spiracles of *Calandra*. In the case of *Tribolium* and *Rhizopertha* a few particles of lamp and carbon blacks and of fine Carborundum have been occasionally found in the spiracles of insects which have been exposed to an excess of the dusts for 24 hours. It is to be noted, however, that the insects were fixed in Carnoy's fluid and cleared before the examination was made and the few particles which were observed may have been carried there by the fixing or clearing agents.

Particle shape.

No very clear evidence that particle shape was an important factor in insecticidal action was obtained although on theoretical grounds it should at least have influenced the quantity of dust adhering to the insects. It has been stated previously that flat particles should adhere better than rounded particles and Zacher (1937) and Heuberger (1942) have reached similar conclusions. The Almicide used contained a large quantity of very thin plate-like aggregates, but perhaps because of their size its adherence was low. Certain kinds of talc also consisted of plate-like particles but they were not found to be more adherent than other materials of similar

particle size range either in the present experiments or, as far as can be judged, in those of Chiu (1939).

A sample of flaky powdered glass and the same material converted into spheres by heat treatment was obtained. Unfortunately the particle size was large and the adherence consequently low so that attempts to measure it were unsuccessful. However, it was found that the sharp flaky form killed insects more quickly than the smooth spherical form and might consequently be regarded as the more abrasive, though the possibility that the particle size distribution was somewhat altered by the heat treatment was not excluded.

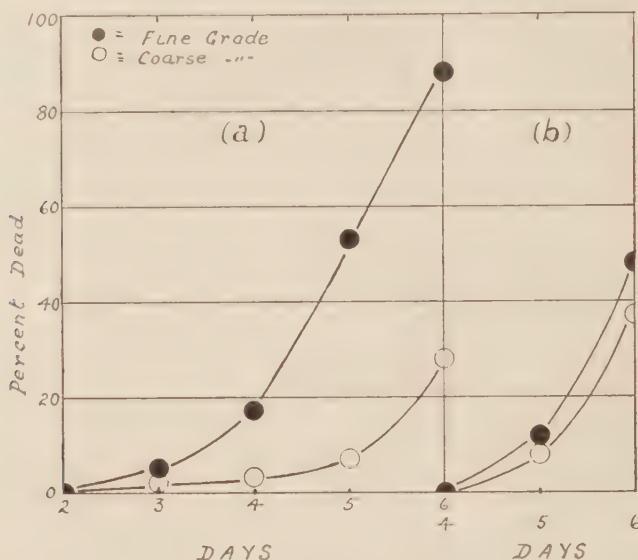


Fig. 12.—*Calandra* was killed more rapidly by (a) fine slate and (b) talc than by coarser grades. The samples were prepared by elutriation and had the following size ranges (diameters).

Slate, coarse 5–170 microns, fine <0.5–4 microns; talc, coarse 10–100 microns, fine <0.5–15 microns. Tested at 25°C. and 60 per cent. R.H. Beetles 14–28 days old.

Each point in the average of three determinations.

TABLE XI.

Particle Shape	Percentage kill of <i>Rhizopertha</i> observed after days			
	4	5	6	7
Flat	0	11	26	37
Round	0	2	9	11
Control	0	4	7	12

Rhizopertha was killed more quickly by a glass powder in which the particles were sharp and flat than by another powder in which the particles were round and smooth. Test conditions were 25°C. and 0 per cent. R.H.

Hardness.

The hardest powders are usually the most abrasive and lethal to insects. It has been concluded (Alexander & others, 1944b) that there is a general correlation

between the hardness and the insecticidal effectiveness of non-toxic dusts. The correlation is not complete, however, as various other factors are involved and some materials appear to behave in an anomalous manner. It is evident that the material must be in a sufficiently fine state of division otherwise it cannot exert its abrasive action (p. 27), but even if this condition is fulfilled all the anomalies are not accounted for.

When the lethal action of the powders used in these experiments was examined, it was found that there was a general correlation between hardness and effectiveness. This is evident from several text figures if the hardness data is taken into account and from the summarised data given in Table XII.

TABLE XII.

Powder	Moh's Hardness	Percentage kill observed after days							
		<i>Calandra</i>				<i>Rhizopertha</i>			
		1	2	5	6	1	2	5	6
Carborundum 700	9-10	51	100	100	100	—	—	—	—
Almicide ...	9 approx.	63	95	100	100	100	100	100	100
Neosyl-Silica ...	7 "	19	46	100	100	56	87	100	100
Slate ...	3 "	0	6	92	100	0	13	57	53
Stockalite-Kaolin	2-2.5	0	0	22	59	0	0	27	38
Talc ...	1-1.5	0	0	49	57	0	0	7	10

Moisture content.

When a substance is exposed to damp air, it tends to absorb moisture from the air until an equilibrium is reached at a value which depends upon the substance and the relative humidity of the air. This relationship has been studied for a variety of materials under carefully controlled conditions (Wilson & Fuwa, 1922) but for the

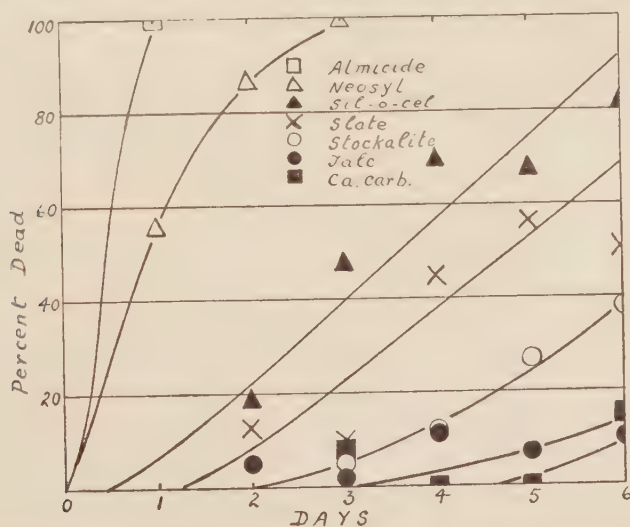


Fig. 13.—Dusts in equilibrium with the same relative humidity were not equally effective in bringing about death of *Rhizopertha* 14-28 days old by desiccation in the continued contact test. Temperature 25°C. 60 per cent. R.H. Each point is the average of three determinations.

present purposes it seemed sufficient to allow a thin layer of the powder to stand in a petri dish kept at the desired relative humidity and to determine the moisture content gravimetrically after several weeks.

A variety of dusts was stored at 25°C. and 60 per cent. relative humidity for several weeks and the rates at which each brought about deaths by desiccation were compared by the continuous contact procedure. Some typical results are shown in detail in figs. 1 and 13, while Table XIII gives a general summary. From the summary it may be seen that the dusts retained the same relative order of effectiveness when compared against the four species, although there were minor variations among the less active dusts between which the differences were small.

TABLE XIII.

Order of effect- iveness		<i>Tribolium</i> R. Humidity		<i>Rhizopertha</i> R. Humidity		<i>Calandra</i> R. Humidity		<i>Ptinus</i> R. Humidity	
		0%	60%	0%	60%	0%	60%	0%	60%
1st	...	Almicide	Almicide	Almicide	Almicide	Almicide	Almicide	Almicide	Almicide
2nd	...	Neosyl	Neosyl	Neosyl	Neosyl	Neosyl	Neosyl	Neosyl	Neosyl
3rd	...	Slate	Slate	Slate	Slate	Slate	Slate	Slate	Kaolin
4th	...	Kaolin	Kaolin	Kaolin	Kaolin	Cal. carb.	Cal. carb.	Kaolin	Slate
5th	...	Talc	Talc	Talc	Talc	Kaolin	Talc	Talc	Talc
6th	...	Cal. carb.	Cal. carb.	Cal. carb.	Cal. carb.	Talc	Kaolin	Cal. carb.	Cal. carb.

The lethal effect of a non-toxic dust depends on its ability to produce desiccation and its action is therefore more rapid under dry conditions (Germar, 1936). This can be seen to hold for a variety of dusts acting on *Tribolium* (fig. 14a and b).

When insects were exposed to dusts which had been brought into equilibrium with saturated air and the test was conducted in a saturated atmosphere even the most active dusts were without effect. Under these conditions no kills were recorded and it seems safe to conclude that the dusts were indeed entirely non-toxic and killed insects only by their desiccating action.

Batches of *Tribolium* were exposed to Almicide, slate and calcium carbonate dusts and the weight lost and the percentage killed were determined for each at intervals. The most active dusts caused the most rapid loss of weight and the highest percentage to be killed for the lowest average loss of weight (Table XIV and fig. 15).

TABLE XIV.

A test on *Tribolium* at 0 and 60 per cent. R.H. and 25°C.

Percentage killed at time (hours) stated												
Total wt. lost %	0% R.H.						60% R.H.					
	Almicide		Slate		Calc. carb.		Almicide		Slate		Calc. carb.	
	Kill	Time	Kill	Time	Kill	Time	Kill	Time	Kill	Time	Kill	Time
10 ...	12	4	0	16	0	53	10	6	0	46	0	104
20 ...	90	10	15	40	0	100	66	11	1	100	0	230
30 ...	99	16	33	60	18	164	96	20	12	170	—	—
40 ...	—	—	55	79	40	178	—	—	—	—	—	—

From the results on batches of *Tribolium* given in fig. 15, it would be inferred that when individual insects died in Almicide they did so after losing much less weight (water) than insects which died in slate dust. In fact, in Almicide, a kill of 100 per cent. was recorded when on the average about 30 per cent. weight had been lost, while in slate the loss would have been over 60 per cent. wt. when the same percentage had been killed. When the weights of individual *Calandra granaria* were followed, however, it was shown that, in slate, death took place after 98 hours at an average wt. loss of 30.9 per cent. In Almicide it took place 34 hours at an average loss of 25.3 per cent. wt. In fact, therefore, individual insects died at very

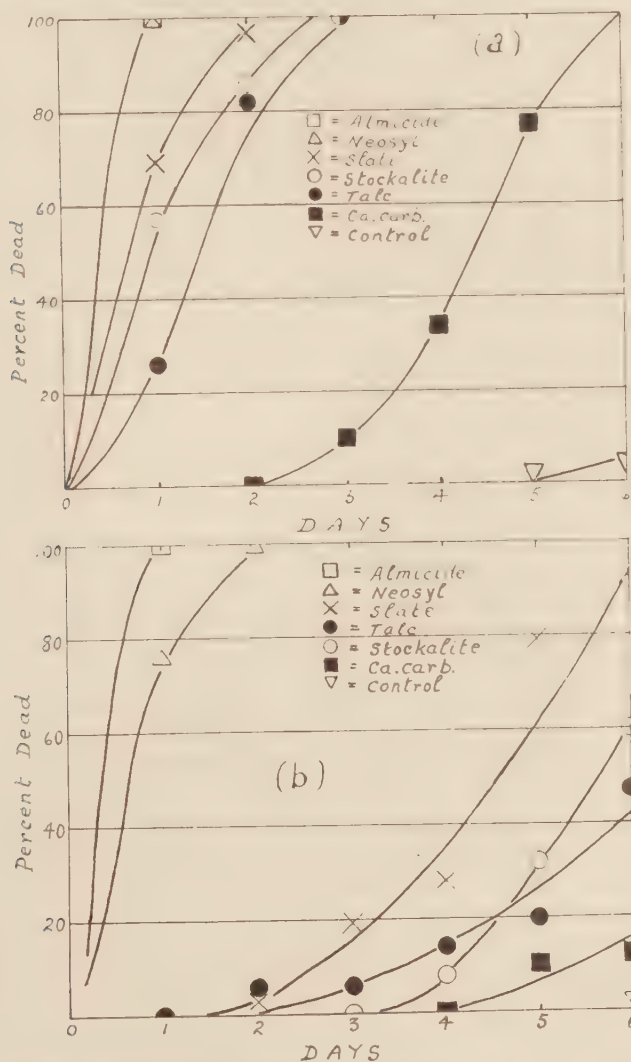


Fig. 14.—Non-toxic dusts killed *Tribolium* more rapidly at (a) low than at (b) high humidities. Continued contact at 25°C. The beetles were between 14 and 28 days old at the beginning of the test and every point represents the average of three tests using 20 beetles for each.

nearly the same weight loss in Almicide and in slate. Probably a slightly greater weight was lost at the time of death in slate dust because more food reserves had been used and more excrement had been voided during the longer period intervening before death. This practical equality of the weights lost when a given percentage of the insects had been killed in Almicide and slate was again entirely masked if

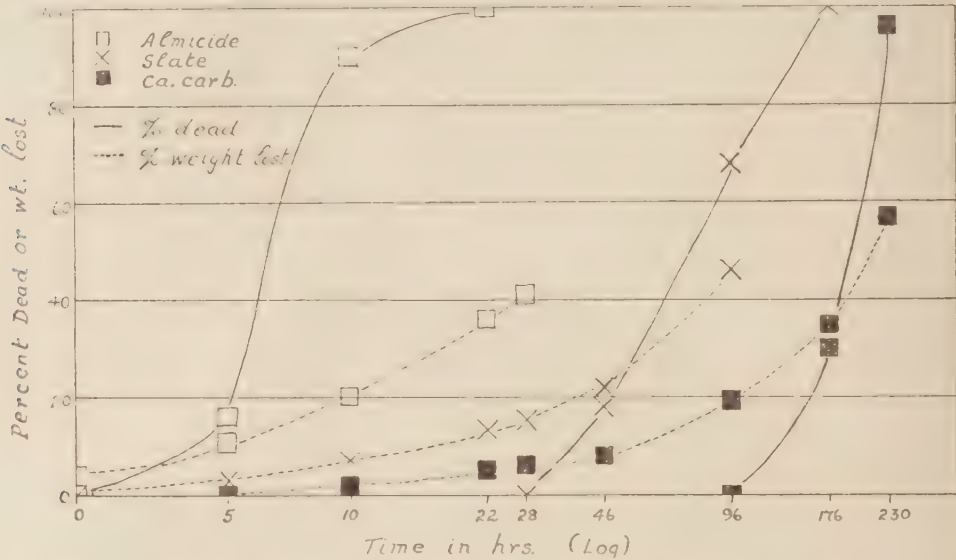


Fig. 15.—*Tribolium* exposed to Almicide, slate and calcium carbonate at 25°C. and 0 per cent. R.H. The more abrasive the dust the more rapid was the kill and the less the total weight lost for a given kill.

average figures were taken from the same experiment. For example, at an average weight loss of approximately 21 per cent., the kill in slate was only 20 per cent. as compared with 60 per cent. in Almicide. The results of the experiments with *Calandra* are given in Table XV and other aspects of this experiment and one on *Tenebrio molitor* are illustrated in fig. 16.

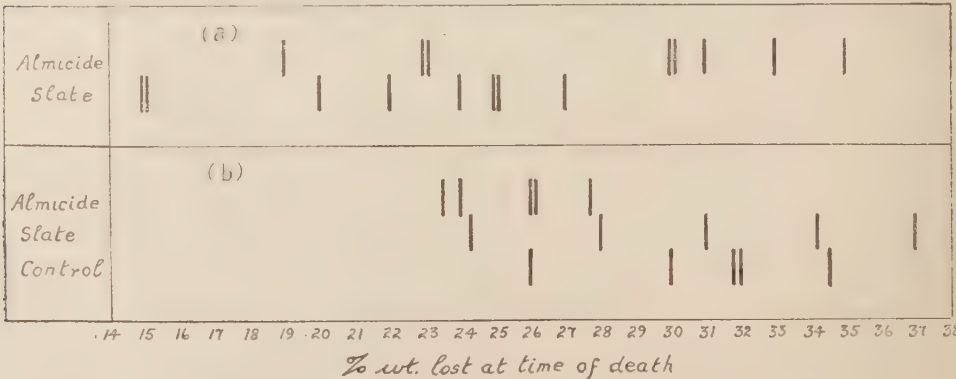


Fig. 16.—Experiments with individual (a) *Tenebrio* and (b) *Calandra* to show that the percentage weight lost at the time of death was very nearly the same whether the insects were untreated or were exposed to Slate or Almicide. The time before death supervened was, of course, longest in the untreated insects and shortest in Almicide. Conditions 25°C. and 60 per cent. R.H.

TABLE XV.

Showing the weights lost by individual *C. alaudra* unfluted and exposed to either slate dust or Alnicole. The weights lost at the time of death (the mean of the last live weight and first dead weight) and the times at which the deaths occurred are also shown. The times, read horizontally in the main body of the table, do not correspond with each other as will be realised by reference to the times of death at the bottom of the table. The ——— indicates that the beetles are dead.

Time	Control				Slate Dust				Almicide												
	Weight lost %		Av. wt. lost %	Dead %	Weight lost %		Av. wt. lost %	Dead %	Weight lost %		Av. wt. lost %	Dead %									
Increasing time	18.1	18.0	15.5	20.8	18.3	18.0	0	2.5	2.2	1.7	2.9	3.5	2.6	0	2.9	10.8	14.7	7.9	11.3	9.5	0
	22.4	23.3	18.9	25.8	24.0	22.9	0	13.3	10.9	14.7	10.0	18.9	15.6	0	8.1	21.6	25.2	16.6	23.5	19	20
	26.8	28.6	21.6	29.6	26.6	26.6	20	18.3	17.5	23.2	17.3	20.7	21.2	20	9.6	24.4	27.2	18.0	25.7	21	60
	29.4	36.0	25.7	33.8	33.6	31.7	60	30.8	26.2	32.8	25.9	40.5	31.2	40	15.4			25.2			60
	34.4		29.7	41.0	43.4		80	37.5	32.8	43.2	36.0	50.4	39.9	80	21.3			30.2			80
			33.0				80		41.0					100	30.9						100
			35.8				100														
% loss at death	31.9	26.0	34.4	31.7	30.1	30.8	100	34.2	36.9	28.0	31.0	24.3	30.9	100	26.1	23.0	26.2	27.7	23.5	25.3	100
Time of death (hrs.)	180	132	228	156	156	170		109	132	83	109	58	98		58	25	25	42	18		34

From the data given, it can be seen that the insects appeared to withstand a greater loss of moisture when it was lost slowly in slate because the time range over which death occurred was much wider than in Almicide. The beetles which died first continued to lose weight throughout the experiment and, having more time in which to do it, made a larger contribution to the total weight lost than was possible in the case of Almicide which acted quickly. This effect may be further accentuated if, as sometimes happens, the rate at which weight is lost is more rapid after death (Wigglesworth, 1944).

It is well known that insects living on dry food obtain water by oxidising some of the products of digestion and it seemed probable that when subjected to a loss of water they might oxidise food reserves for the same purpose. In quickly lethal dusts, such as Almicide, there might be insufficient time for this process to come into action, but in the more slowly acting state it should be apparent. In fact, there was no evidence that the dry weight of beetles dying of desiccation decreased more rapidly than that of control insects. A steady decline occurs, however, and, as suggested, this probably partly explains the greater weight loss at the time of death observed in insects killed in the more slowly acting dusts (p. 32).

TABLE XVI.

Time (hr.)	Almicide		Slate dust		Control	
	Wt. loss %	Dry wt. %	Wt. loss %	Dry wt. %	Wt. loss %	Dry wt. %
0 ...	0	52	0	53	0	51
10 ...	22	52	0	53	0	51
20 ...	27	49	13	49	2.5	52
96 ...	—	—	45	46	8.0	47

The Properties of DDT Dusts in relation to Insecticidal Action.

The action of non-toxic dusts on insects has already been described, and these observations provide the preliminary information which is necessary in order to understand the mode of action of toxic dusts compounded from such non-toxic carriers and toxic ingredients. With these mixed dusts both components may act on the insects independently and the carrier may be expected to modify the effect of the toxic compound in one way or another.

Preparation of the DDT dusts.

Two methods were used to prepare the DDT dusts :

Solvent application.—A solution of 10–20 per cent. w/v DDT in benzene was prepared, and this was added to the powder in such a volume as to give the required DDT content to the dry dust. When necessary, in order to distribute the DDT uniformly, the concentrated solution was diluted with sufficient benzene to wet the powder thoroughly. After careful mixing the benzene was evaporated on a water

bath with continuous stirring, and the process was completed by about two hours further warming in an oven at 105°C., by which time the dust should be free from the odour of benzene. It was cooled, lightly ground in a ball-mill and sieved through a 30 mesh sieve (BSS 481) on to a paper tray on which it was allowed to condition in the constant temperature room kept at 60 per cent. R.H.

A modification of this type of dust was prepared by making a concentrated mixture of the carrier with DDT by the method described and diluting this with the plain carrier to give the required final concentration.

Simple mixing.—A few dusts were made by mixing and grinding the required quantities of DDT and carrier together in a simple ball mill consisting of $\frac{1}{2}$ -inch ball bearings in a 6–10 oz. screw-topped bottle made to revolve in a "Meccano" frame-work. Under these circumstances the grinding action which took place was probably rather slight and the size and number of particles of DDT in the final mixture was considerably influenced by those of the stock DDT.

The action of the DDT dusts on insects.

Experiments made to investigate the action of dusts containing DDT are described in the following sections.

TABLE XVII.

Comparison of dusts at 25°C. and saturated humidity by the continued contact procedure.

Dust : 5% w/w DDT in	Insect	Percentage kill observed after days					
		1	2	3	4	5	6
Almicide ...	<i>Calandra</i> 1st exp. ...	0	12	40	53	57	84
Kaolin ...		0	15	24	59	83	87
Slate ...		2	16	29	49	68	77
Talc ...		0	15	15	35	68	68
Silica ...		0	3	21	32	58	70
Calc. carb. ...		0	7	34	45	54	85
Control ...		0	0	0	0	0	0
Almicide ...	<i>Calandra</i> 2nd exp. ...	0	1	20	41	61	85
Kaolin ...		0	2	21	47	65	89
Talc ...		0	2	17	36	55	81
Silica ...		0	5	20	41	59	77
Calc. carb. ...		0	0	18	39	56	79
Control ...		0	0	0	0	0	0
Almicide ...	<i>Tribolium</i> 1st exp. ...	0	3	5	44	77	80
Kaolin ...		0	5	10	42	72	83
Talc ...		0	10	23	47	68	87
Silica ...		0	3	5	32	58	80
Control ...		0	0	0	0	0	0
Almicide ...	<i>Tribolium</i> 2nd exp. ...	0	5	15	41	70	84
Kaolin ...		0	8	22	43	70	93
Talc ...		0	13	30	47	67	83
Silica ...		0	13	22	45	72	88
Control ...		0	0	0	0	0	0

Each result is the average of three tests using altogether 60 insects.

Except at saturated humidities the non-toxic carriers act on the insects and may produce death through desiccation. Therefore, in order to find whether DDT is equally active at the same percentage weight concentration in all carriers, the tests must be conducted at 100 per cent. relative humidity with dusts conditioned to this humidity. With the desiccation factor eliminated the dusts might still not be equitoxic as has been suggested in the theoretical section. For example, an increase in the voluminosity of the carrier might be equivalent to dilution and when abrasion occurs this might facilitate the entry of the DDT. The results obtained following the continued contact procedure did not support these suggestions within the limit of sensitivity of the test and from the results set out in Table XVII it can be seen that there was no significant difference in the toxicity of 5 per cent. wt/wt mixture of DDT in six carriers which differed widely in properties. However, it so happens that the two most voluminous dusts are also the most abrasive and it might be suggested that the two opposing effects cancel each other out though it would be a coincidence if they did so almost exactly in both cases. On the whole, therefore, it seems safe to conclude that, when made on a weight basis and tested as described, DDT is not less effective in a voluminous carrier than in a dense dust and that abrasive dusts (which have been shown to retain their abrasive properties at high humidities) do not facilitate the entry of DDT.

In several tests by the discontinued contact procedure using *Calandra* and *Rhizopertha*, the DDT dusts based on various carriers were again almost equitoxic (Table XVIII). This is surprising as large differences in the quantity adhering have been demonstrated and a correlation between the gross quantity adhering and the observed kill would be expected. This subject is discussed further on p. 45.

TABLE XVIII.

Comparison of dusts against *Calandra* at 25°C. and saturated humidity by the discontinued contact procedure.

Dust 5 per cent. w/w DDT in	Percentage kill observed after days				
	2	3	4	5	6
Almicide	3	20	28	43	70
Kaolin	1	11	27	45	70
Talc	2	14	26	45	60
Silica	4	15	31	51	66
Calc. carb.	0	4	24	41	60

Each result is the average of four tests using, in all, 80 insects 24-38 days old.

If, instead of conducting the comparisons between DDT in various carriers at saturated humidity, the insecticidal tests were made at 60 per cent. R.H., then the abrasive action of the carriers was added to the toxic effect of the DDT and the mixtures based on the most abrasive carriers were the most effective. Results obtained under these conditions, by the continued contact method, with the four test beetles are shown in fig. 17 a-d.

By the discontinued procedure the same effects were evident with *Calandra*, *Rhizopertha* and *Ptinus*—that is the most abrasive dust made the most effective carrier (fig. 18a and b). In the case of *Tribolium*, however, the small quantity adhering to this smooth beetle sometimes became the limiting factor and DDT in Almicide which adheres poorly is less effective than the more adherent DDT in Neosyl (Table XIX). In the same way, under these circumstances, Neosyl is more effective than pure Almicide which is otherwise not the case. In both discontinued and continued methods with abrasive carriers and insects of low sensitivity to DDT (e.g. *Ptinus*) the effect of DDT in the mixture may be completely masked by the desiccating action of the dusts (see p. 45).

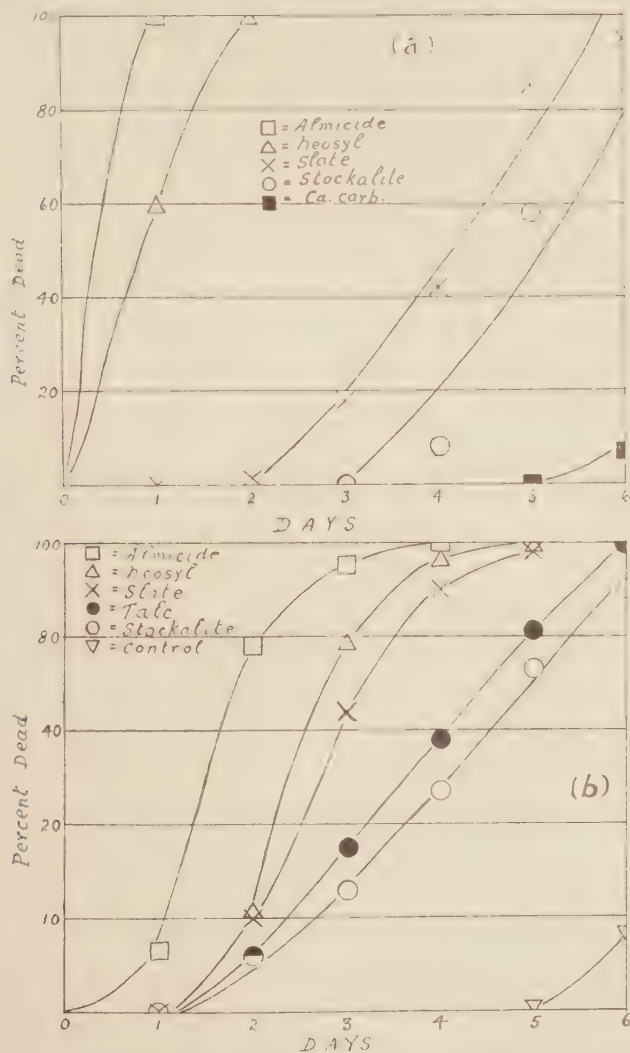


Fig. 17, a, b.—By the continued contact method at humidities below saturation, toxic dusts were more effective when compounded with an abrasive carrier than with a non-abrasive carrier. (a) *Tribolium*, (b) *Calandra* 14–28 days old tested at 25°C. and 60 per cent. R.H. Each point was determined by averaging the results of three tests with 20 beetles.

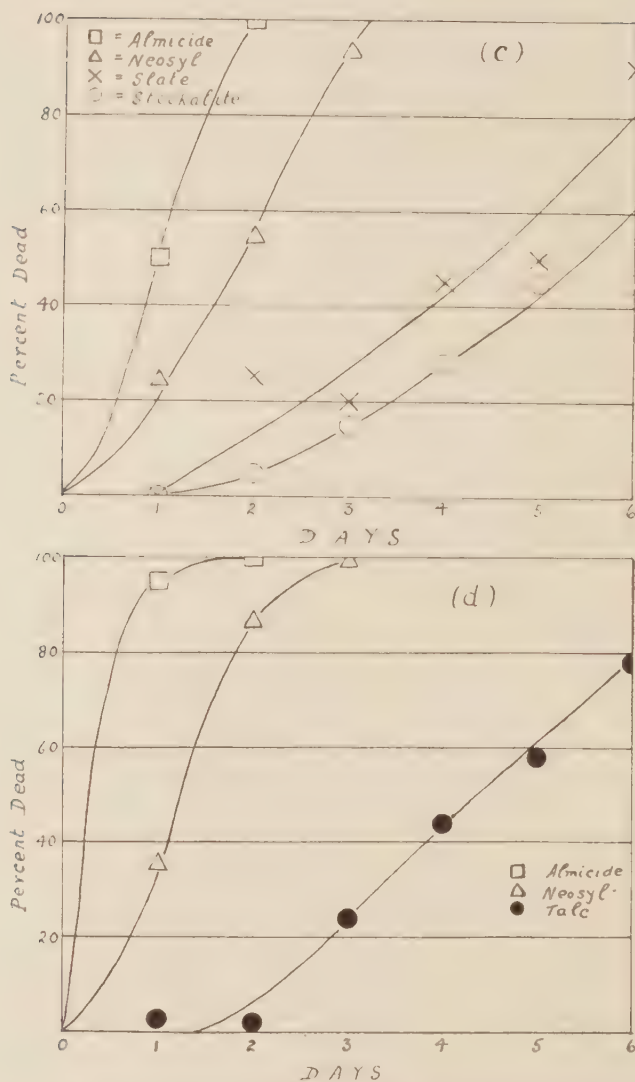


Fig. 17, c, d.—By the continued contact method at humidities below saturation, toxic dusts were more effective when compounded with an abrasive carrier than with a non-abrasive carrier. (c) *Rhizopertha* and (d) *Ptinus* 14–28 days old tested at 25°C. and 60 per cent. R.H. Each point was determined by averaging the results of three tests with 20 beetles.

TABLE XIX.

Dust	Quantity found adhering mg. dust/gm. insects	Percentage of <i>Tribolium</i> killed after days				
		2	3	4	5	6
Almicide + 5 per cent. DDT	<1.0	0	0	1	6	7
Neosyl + 5 per cent. DDT	4.0	1	4	16	47	75

Each figure represents the percentage kill on the day indicated and is the average of four determinations with twenty insects. Temperature 25°C., R.H. 60 per cent.

If a highly abrasive non-toxic dust such as Almicide or Neosyl acts upon insects, it may kill them much more quickly (by desiccation) than pure crystalline *p.p.* DDT as some preliminary experiments with *Rhizopertha* and *Tribolium* by the continuous contact procedure show (Table XX).

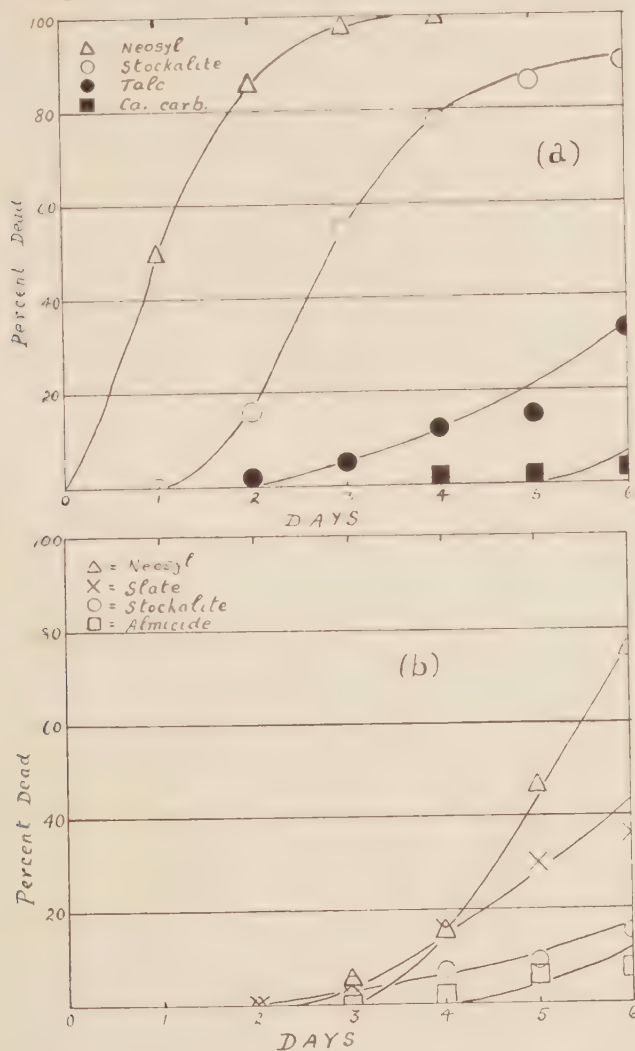


Fig. 18.—By the discontinued contact method, at 25°C. and 60 per cent. R.H., toxic dusts (10 per cent. w/w DDT) provided they adhered were more toxic when compounded with an abrasive carrier than with a non-abrasive carrier, because in the former case the dusts killed the insects by toxic action and desiccating action (a) *Ptinus*. However, if the adherence of a dust was very low it may appear to be ineffective though in fact highly abrasive, e.g. Almicide/DDT on (b) *Tribolium*.

The results given in Table XX show that insects exposed to a mixed DDT carrier dust might be killed largely by the DDT or by desiccation. As the humidity at which the tests were conducted was reduced, the effect of the DDT remained constant but the desiccating action of the carrier became progressively greater.

TABLE XX.

The effect of various dusts on *Rhizopertha* and *Tribolium* at 25°C. and 60 per cent. R.H.

(a) Dust	Percentage of <i>Rhizopertha</i> killed after days					
	1	2	3	4	5	6
Almicide-alumina	100	—	—	—	—	—
Neosyl-silica	56	87	100	—	—	—
Pure DDT	0	10	30	36	60	75
Stockalite-kaolin	0	5	5	12	26	38
Calcium carbonate	0	0	0	0	0	0
Control	0	0	0	0	0	0

(b) Dust	Percentage of <i>Tribolium</i> killed after days					
	1	2	3	4	5	6
Almicide-alumina	100	—	—	—	—	—
Neosyl-silica	100	—	—	—	—	—
Pure DDT	30	90	100	—	—	—
Slate dust	5	30	70	85	100	—
Calcium carbonate	0	0	0	0	0	0
Control	0	0	0	0	0	0

Each figure is the average of three determinations.

The addition of DDT to a non-toxic dust therefore increased its killing power when its abrasive properties were poor and or when the prevailing humidity was rather high, for example, talc at 60 per cent. R.H. With an abrasive dust such as Neosyl on the other hand, the effect of adding DDT at the same humidity was slight while in the case of the very abrasive Almicide the addition of DDT decreased the rate of kill. All these results are illustrated with *Calandra* in fig. 19.

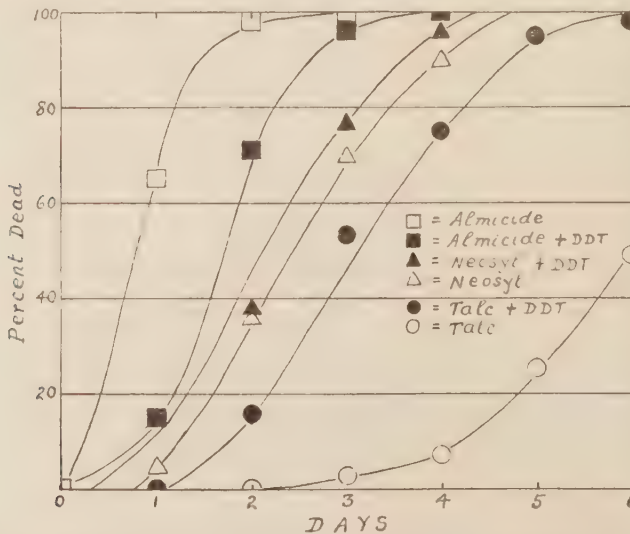


Fig. 19.—DDT decreases the rate of action of Almicide, has little effect on Neosyl and greatly increases that of talc. *Calandra* 14–20 days old at 25°C. and 60 per cent. R.H. Each point was based on three tests with 20 insects.

An attempt was made to determine why (as noted in the previous paragraph) DDT reduced the rate of action of Almicide on *Calandra*. In a preliminary experiment, it was found that the insects in Almicide lost weight more quickly than those in Almicide containing 5 per cent. w/w DDT whether the mixture was made by solvent application or by simple mixing (Table XXI). It seemed probable, therefore, that the observed effect was not due to a reduction in the abrasiveness of Almicide since, although this might have occurred if the particles became coated with DDT during solvent application it probably could not have occurred as a result of simple mixing. The difference between the effect of the Almicide alone and with DDT was confirmed in a more detailed experiment (Table XXII).

TABLE XXI.

Calandra exposed to dusts at 25°C. and 60 per cent. R.H.

Time in hours	Almicide		Dust applied Almicide and DDT from solvent		Almicide and DDT mixed in	
	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.
6	4	0	5	0	5	0
30	26	72	23	47	22	37

TABLE XXII.

Calandra exposed to dusts at 25°C. and 60 per cent. R.H.

Time in hours	Dust applied							
	Almicide		Almicide and DDT from solvent		Almicide and DDT mixed in		Control	
	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.
5	2.4	0	2.0	0	1.9	0	0.9	0
11	7.1	1	5.6	0	5.6	0	2.1	0
23	14.7	20	12.4	7	11.4	7	5.3	0
29	18.8	44	15.4	30	14.8	25	—	—
35	22.7	76	19.1	67	17.1	63	6.3	0
47	29.6	99	24.8	97	22.7	93	7.8	0

It may be concluded that when DDT was added to Almicide it reduced the rate at which water was lost from the insects. For a given loss of weight, however, the kill was greatest in the presence of DDT.

It has already been shown that DDT acted comparatively slowly on the beetles used as test insects whereas Almicide was much more rapid in action (p. 41). It might be suggested, therefore, that the effect of adding DDT to Almicide was merely that of diluting a highly active abrasive. If this was true a comparable effect ought to have been produced by any non-toxic diluent, and Almicide was therefore "diluted" with 5 per cent. DDT, talc or calcium carbonate. It was found that calcium carbonate, perhaps owing to its relatively large particle size, was almost without effect and that talc reduced the rate of action at 25°C. and 60 per cent. R.H., but to a lesser extent than DDT (Table XXIII). It may be concluded, therefore, that the dilution effect does not entirely account for the action of DDT on Almicide.

TABLE XXIII.
Calandra granaria.

Exp. No.	Percentage killed after days in dust at 25°C. and 60 per cent. R.H.											
	Almicide			Almicide and 5 per cent. w/w DDT			Almicide and 5 per cent. w/w Talc.			Almicide and 5 per cent. w/w Calc. Carb.		
	1	2	3	1	2	3	1	2	3	1	2	3
1	15	59	94	11	40	84	5	41	90	4	46	92
2	4	59	95	1	41	84	2	52	96	1	56	98
3	5	40	90	1	31	77	2	35	87	1	45	90
4		59	89		39	75		58	88		66	90
5		67	94		53	89		55	85			
Av.	8	57	92	4	41	82	3	48	89	2	53	92

Any doubts as to whether the dilution effects just discussed entirely explained the action of DDT on the desiccating properties of Almicide were dismissed by the observation that a pre-treatment of the insects with DDT for one hour before exposing them to Almicide also caused a marked reduction in the rate of death. It seemed possible that this was partly due to the DDT mechanically preventing the Almicide from reaching the sites where abrasion occurred since pre-treatment with talc produced almost as much effect. However, exposing the insects to a film of DDT (1,000 mg/sq. ft.) on filter paper acted in the same way. Under the latter circumstances the pre-treatment lasted for 24 hours and the reduction in the rate of the kill resulting from subsequent exposure to Almicide was even more marked. It seems safe to conclude that the DDT modified the behaviour of the insects in such a way as to have reduced the amount of abrasion which occurred. When exposed to an Almicide DDT mixture there was little time for this action to take place, with the one-hour pre-treatment with DDT there was more opportunity, while with the 24-hour pre-treatment there was even more time and under these latter circumstances the maximum effect was observed (Table XXIV).

TABLE XXIV.

Insecticide	Percentage of insects killed after days at 25°C. and 60 per cent. R.H.		
	2	3	4
Almicide only	67	94	99
Almicide+5 per cent. pure DDT	53	89	99
Almicide after pure DDT (1 hr.)	43	90	97
Pure DDT only (1 hr.)	10	20	60
Almicide after Talc (1 hr.)	54	93	100
Talc only (1 hr.)	0	0	7
Almicide after DDT film (24 hrs.)	19	35	62
DDT film only (24 hrs.)	19	37	72

Each figure was the average of four determinations using 20 *Calandra* for each.

Since the beetles were less sensitive to the desiccating action of Almicide and lost weight more slowly in the presence of DDT it seemed possible that pre-treatment with DDT might also reduce the rate at which water was lost from beetles exposed to dry air. This would have occurred, for example, if the DDT caused the spiracles

to close or in some way decreased the evaporation from the insect through an effect on activity or cuticle permeability. In fact there was no evidence that beetles which had been exposed on pure DDT lost weight any more slowly in dry air than untreated insects. The DDT seems therefore only to affect the desiccating action of Almicide and not the general tendency of the insects to lose water (Table XXV).

TABLE XXV.

Calandra granaria after pre-treatment for one hour with pure DDT at 25°C. and 0 per cent. R.H.

Time (hrs.)	Insects pre-treated with DDT		Control Insects	
	Wt. lost per cent.	Kill per cent.	Wt. lost per cent.	Kill per cent.
3	1.48	—	1.35	—
7	3.18	—	3.15	—
24	10.10	—	10.20	—
33	13.70	—	14.50	—
48	22.10	5	21.70	10
72	28.20	100	28.40	100

From a consideration of their physical properties it seemed probable that some of the carrier DDT dusts would adhere to the insects in larger quantities than others and in consequence the most adherent would be the most lethal. On the other hand the test insects are not very sensitive to DDT so that the effect might not be apparent. *Calandra granaria* was the most suitable insect on which to test these possibilities since it was more susceptible to DDT than *Rhizopertha* or *Ptinus* and more dust adhered to it than to *Tribolium*. Consequently batches of *Calandra* were treated by the discontinued contact procedure and the dusted insects were then kept at 100 per cent. R.H. and 25°C. From the results given in Table XXVI it can be seen that six times as much of one dust as of another may adhere without causing any appreciable difference between the percentages of insects killed and it must be concluded that much of the DDT in the more adherent dusts was located on areas where it could not become effective (Table XXVI).

TABLE XXVI.

No.	Dust 10 per cent. DDT in	Quantity found adhering mg. dust/g. of insect after (hr.)				Percentage of <i>Calandra</i> killed after days		
		<0.25	24	48	120	4	5	6
1	Almicide ...	9.2	1.5	0.75	0.0	0	29	61
	Stockalite ...	11.5	1.5	0.75	0.0	0	27	63
	Slate ...	16.8	6.2	4.6	3.1	0	17	39
	Neosyl ...	10.8	3.1	1.50	0.75	0	28	67
	Sil-o-cel ...	6.2	1.5	0.75	0.75	0	33	60
	Cal. carb. ...	27.8	24.6	18.5	13.9	0	31	60
	Control ...	—	—	—	—	0	2	2
2	Almicide ...	6.6	1.1	—	0.7	46	49	71
	Stockalite ...	11.8	1.5	—	0.7	59	61	83
	Slate ...	29.8	13.3	—	11.7	69	71	84
	Neosyl ...	5.9	1.5	—	0.7	64	66	86
	Sil-o-cel ...	5.9	0.7	—	—	60	62	84
	Calc. carb. ...	31.0	26.6	—	19.2	69	72	87
	Control ...	—	—	—	—	1	1	1

The percentage kills were each based on three lots of 20 *Calandra* kept at saturated humidity and 25°C. after exposing to the dusts.

It has been stated (p. 22) that the quantity of a given dust adhering to beetles after removing the excess of dust tended to reach a constant value and that this value was not easy to reduce by further vibration. In order to investigate the effects of variations in the quantity of any one dust adhering to beetles on the resultant kills, it was therefore necessary to take special measures to remove the adherent particles. This was achieved by sucking air over the insects, either in bulk when they were on a sieve, or individually, in which case the dust could be removed from selected areas only. Both methods were used and it was shown clearly that removing the dust reduced the kill (Table XXVII).

Calcium carbonate was used as the carrier as its mixture with DDT was more adherent than the other dusts and therefore allowed greater variations to be made in the doses. It must be concluded that for one carrier the quantities adhering were related to the kills but that when different carriers were involved the DDT was not equally available from each and that in this case the quantities present were not related to the kills. One of the consequences of this conclusion is that chemical estimations of the amounts of insecticides adhering to insects in different carriers will not provide reliable information about the relative biological effectiveness of the preparations.

TABLE XXVII.

Calandra dusted with 10 per cent. DDT in calcium carbonate.

Exp. No.	Treatment	Quantity of dust left adhering mg./gm.	Percentage of insects killed after days				
			2	3	4	5	6
1	Sieved only	36.8	3	37	83	93	97
	Sieved and brushed ...	18.4	7	33	67	90	93
	Sieved and vacuumed ...	6.1	0	20	27	33	37
2	Sieved only	33.8	0	3	40	80	83
	Sieved and bulk vacuumed	18.4	0	3	27	53	60
	Sieved and vacuumed individually	9.3	0	5	5	15	20

The test insects were kept at 25°C. and 100 per cent. R.H. and each figure represents the average of three tests, using altogether 60 insects.

It might be supposed that a dust which effectively abraded the cuticle and damaged the outer impervious layer in such a way as to render the insect more permeable to water would also favour the entry of insecticides. Wigglesworth (1945) has in fact shown that the dorsal cuticle of a *Rhodnius* nymph was made more permeable to nicotine and rotenone by first rubbing it with an abrasive but recognised that the effect might not occur in practice. Following this suggestion Hunt (1947) reported that abrasive carriers facilitated the entry of cryolite (sodium fluoaluminate, Na_3AlF_6) and rotenone into the larvae of the Mexican bean beetle but other explanations of the results which he obtained do not seem to be excluded.

In order for an abrasive dust to increase the rate of penetration of an insecticide under practical conditions it must either (a) increase the permeability of the cuticle in areas through which the insecticide normally passes or, (b) render permeable areas which are normally almost impermeable. In either case the permeability must be enhanced at the site of abrasion by a significant amount in relation to that previously occurring there. By directly comparing abraded and non-abraded regions of the rather impermeable dorsal cuticle and confining the insecticide to those regions only Wigglesworth (1945) emphasised the contrast and demonstrated, as might be expected, an increase in permeability following abrasion under these conditions.

A series of experiments were planned in order to determine whether there was any evidence that in practice DDT or rotenone killed the test beetle more readily when mixed with abrasive rather than non-abrasive carriers. The tests were conducted at saturated humidity to eliminate the desiccating action of the abrasive carriers. Under these conditions the non-toxic dusts were without effect but were still abrasive, as was demonstrated by examining beetles which had been exposed to the dust by the silver staining method. It might be argued that coating an abrasive dust with DDT by solvent application (p. 36) would cause it to lose its abrasive properties. In order to overcome this objection DDT dusts were made up by diluting a concentrated DDT dust with more of the untreated non-toxic carrier or by simply mechanically mixing in the DDT. In no case was there any evidence that either DDT or rotenone was more effective in an abrasive Almicide and Neosyl than in a non-abrasive (Stockalite and talc) carrier (Tables XXVIII(a) and (b) and XXIX).

TABLE XXVIII (a).

Calandra continuously exposed to a dust coated with DDT applied from a solvent.

Dust 5 per cent. w/w DDT in	Percentage killed after days					
	1	2	3	4	5	6
Almicide	0	19	33	58	66	76
Neosyl	0	18	56	64	78	90
Stockalite	0	14	52	78	89	96
Talc	0	22	62	78	80	95
Control	0	0	0	0	0	0

TABLE XXVIII (b).

Calandra continuously exposed to dust with DDT applied by simple mechanical mixing.

Dust 5 per cent. w/w DDT in	Percentage killed after days					
	1	2	3	4	5	6
Almicide	0	7	19	39	50	72
Neosyl	0	10	20	40	77	92
Stockalite	0	8	26	42	57	88
Talc	0	14	40	65	60	81
Control	0	0	0	0	0	0

Each percentage is based on three determinations with 20 insects at 25°C. and 100 per cent. R.H.

TABLE XXIX.

Tribolium continuously exposed to dust carrying DDT.

Dust 5 per cent. w/w DDT on	Percentage killed after days					
	1	2	3	4	5	6
Almicide	0	4	13	46	78	87
Neosyl	0	5	10	32	64	82
Stockalite	0	4	13	45	75	93
Talc	0	8	29	49	71	88
Control	0	0	0	0	0	0

Each percentage was based on nine determinations with 20 insects at 25°C. and 100 per cent. R.H.

The results for 5 per cent. rotenone dusts have not been tabulated since the beetles were very resistant to this insecticide. *Calandra* seems to be the most susceptible species but there was no evidence that its effect was increased by incorporating the rotenone in an abrasive carrier. From the foregoing tests it may be concluded that under the conditions of exposure used (which cannot be regarded as differing in essentials from those which might have occurred in practice) there was no evidence that an abrasive carrier facilitated the penetration of DDT or rotenone. As an explanation of this observation it might be suggested that the sites at which abrasion occurred were in their normal state readily penetrated by the insecticides, or on the other hand, that the abraded areas were relatively unimportant sites of insecticide absorption the main part of which was taking place more rapidly elsewhere.

Three types of DDT dusts were prepared as described (p. 36) in order to determine whether the method used for distributing the DDT in these carrier dusts whether by solvent application or by single mixing, influenced the toxicity. The first preparation was made by adding a solution of DDT in benzene to the dust so as to give the desired concentration, the second by preparing a concentrated mixture in this way and then diluting with more carrier. The third dust was prepared by simply mixing the solid ingredients in a slowly revolving ball mill. The DDT contained crystals up to $400 \times 40\mu$ but the majority were less than $100 \times 10\mu$. It is unlikely that the particle size of the DDT was materially reduced by the light ball milling given.

The three dusts were compared by the continued contact method at saturated humidity. It was found that the dust produced by simple mixing was invariably less effective than those prepared by solvent applications which were approximately equally effective. Typical results obtained with *Calandra* and *Tribolium* are shown in fig. 20a and b. The experiments were repeated with various types of carriers and the results were the same in each case.

The Action of Dusts containing added Oil on the Insects.

Theoretically it seemed possible that adding a mineral oil to a DDT dust might increase its insecticidal effect either by its own toxic action, by facilitating the absorption of DDT, or by increasing the quantity of dust adhering to the insects. It has in fact been shown (Hewlett, 1947) that certain mineral oils killed *Calandra granaria* (though probably by suffocation) and that adding oil to rotenone dusts increased, for unexplained reasons, the effectiveness of the dusts in killing pea aphids on potted plants (Campau & others, 1942; Wilson & Campau, 1943). The latter results may well have been due to either increased adherence or penetration.

Several dusts were tested after adding 5 per cent. v/w of odourless kerosene which was fairly volatile or 5 per cent. v/w of the relatively non-volatile white oil, Shell P. 31 (see David 1946b, Appendix 1, for specifications). The oils were applied from petroleum ether which was subsequently evaporated by airing and warming for a short time. The dusts were then conditioned to 100 per cent. relative humidity for 48 hours. When tested at this humidity and at 25°C. they were entirely without effect on *Tribolium* or *Calandra* after six days. It may therefore be concluded that the oiled dusts were non-toxic.

It might be supposed that an oiled dust would be less capable of abrading an insect than an untreated dust or that, owing to some alterations in its properties, it might fail to reach the sites at which the abrasions normally occur. In fact there was no evidence that oiled silica (Neosyl) prepared as described in the foregoing section was less abrasive and insecticidal than untreated silica (Table XXX).

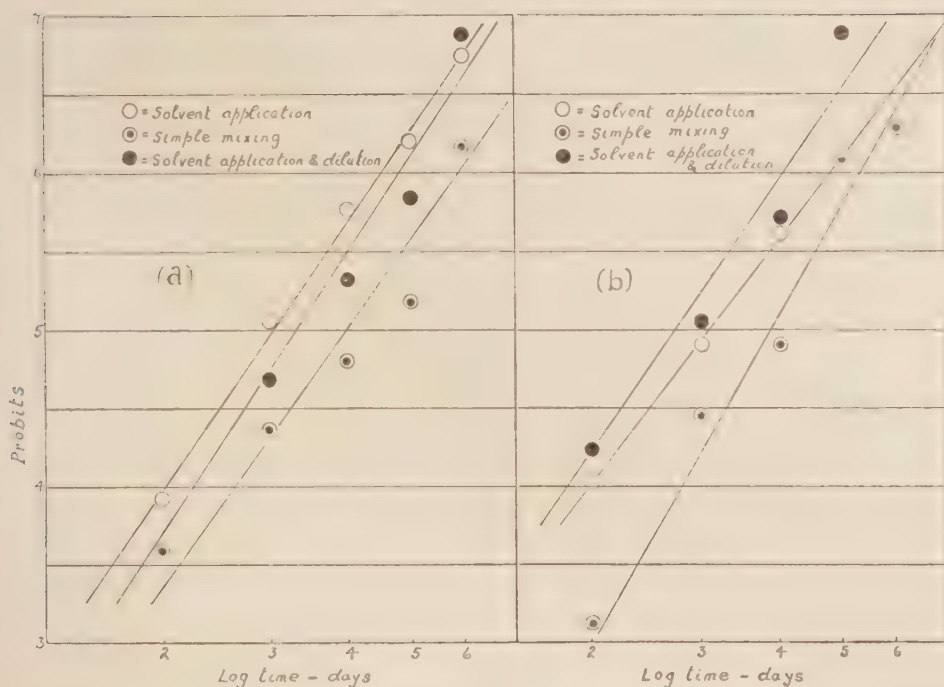


Fig. 20.—Tested on (a) *Calandra* and (b) *Tribolium* a DDT/carrier dust made by simple mixing was found to be less toxic than two dusts made by solvent application methods, which were almost equally effective. Tested at 25°C. and 100 per cent. R.H. Each figure is based on three tests using 20 insects for each.

TABLE XXX.

Dust	Percentage killed after days		
	1	2	3
Neosyl	36	100	—
Neosyl+5 per cent. v/w odourless kerosene	46	98	100
Neosyl+5 per cent. v/w white oil P.31	40	100	—
Control	0	0	0

The test insect was with *Tribolium* at 25°C. and 60 per cent. R.H. The average figures were based on three determinations using 20 insects 21–35 days old for each.

From a variety of experiments conducted with oiled and unoled dusts it was concluded that when tested by continued contact (when only the toxic action and not the adherence of the dusts came into play) oiled dusts were not markedly more toxic than unoled dusts but that occasionally, in the case of insects susceptible to DDT, there was some evidence of an increase in toxic action. Thus oiled and unoled dusts consisting of slate or silica with 5 per cent. w/w DDT were equally toxic to *Rhizopertha* and all almost without effect on *Ptinus* when exposed at 25°C. and 100 per cent. R.H. for six days. Under similar conditions there was some, though not convincing evidence that the oiled dusts were slightly more toxic to *Calandra*.

In a series of experiments with rotenone there was no evidence that dusts containing oil in addition were more toxic. The beetles were, however, very resistant to rotenone, as will be shown later, but in any case the effect of oil could not have been pronounced.

In experiments following the discontinued contact procedure the effect of enhanced adherence was seen in the case of oiled dusts acting on *Tribolium* to which, since the insects were so smooth, very little dust normally adhered. The adherence and the kill were markedly increased in this case while the effect was progressively less apparent with *Calandra* and *Rhizopertha* and negligible with *Ptinus* (Table XXXI).

TABLE XXXI.

Dust kaolin + 5 per cent. w/w DDT	Beetle	Quantity adhering after time (by colour) (mg./g.)			Percentage killed after days				
		Min. 5	Hr. 24	Hr. 48	2	3	4	5	6
Unoled ...	<i>Tribolium</i> ...	4.7	0.0	0.0	0	0	0	0	1
Oiled ...		18.8	5.9	4.7	7	27	59	72	89
Control ...		0.0	0.0	0.0	0	0	0	0	0
Unoled ...	<i>Calandra</i> ...	13.8	3.1	1.5	4	12	13	32	55
Oiled ...		15.4	2.3	2.3	6	21	39	61	71
Control ...		0.0	0.0	0.0	0	0	0	0	0
Unoled ...	<i>Rhizopertha</i> ...	3.2	4.9	4.9	0	4	4	6	6
Oiled ...		3.2	7.3	9.7	0	5	7	9	10
Control ...		0.0	0.0	0.0	0	0	0	0	0
Unoled ...	<i>Ptinus</i> ...	52.3	21.5	13.8	0	0	0	0	0
Oiled ...		52.3	21.5	16.8	0	0	0	0	0
Control ...		0.0	0.0	0.0	0	0	0	0	0

The tests were conducted by the discontinued contact method. Each average figure was the result of three determinations with 20 insects at 25°C. and 100 per cent. R.H. using beetles 16-30 days old.

The Properties of Rotenone Dusts in Relation to Insecticidal Action.

An attempt was made to use rotenone, either in its pure form or as the oleo-resin, as an alternative insecticide to DDT but the beetles proved to be so resistant to its action that no satisfactory results were obtained.

Preparation of the dusts.

The rotenone was added to the dust from a solution in benzene which was subsequently evaporated on a water bath and by two hours oven treatment at 100°C. Alternatively pure rotenone was simply mixed with the carrier dust. In some experiments a Derris oleo-resin, containing 25 per cent. w/w rotenone, was used undiluted and dusts which contained up to 5 per cent. of mineral oil in addition to the rotenone were also tested.

Results of experiments with rotenone dusts.

The four species of beetles proved to be very resistant to rotenone in the pure form and as the oleo-resin. For example, when *Rhizopertha* was exposed to an abrasive carrier and then to pure rotenone for six days none of the beetles was killed and in the case of *Calandra* similarly treated the percentage killed was no higher than that observed in the controls. The tests were conducted at 100 per cent. R.H. and 25°C.

Since it seemed possible that the penetration of the rotenone might be facilitated by oils, about 5 per cent. w/w of various grades were added to the dusts which contained up to 2 per cent. w/w of pure rotenone. None of the oils tested—odourless kerosene, white oil P.31 and a medium lubricating oil—had an appreciable effect and the kills recorded after six days exposure at 25°C. and 100 per cent. R.H. were negligible.

Certain Characteristics of the Test Insects in relation to Dust Action.

It is evident that both the properties of the dusts and those of the insects may vary considerably. Most of the tests hitherto described demonstrate the effects of

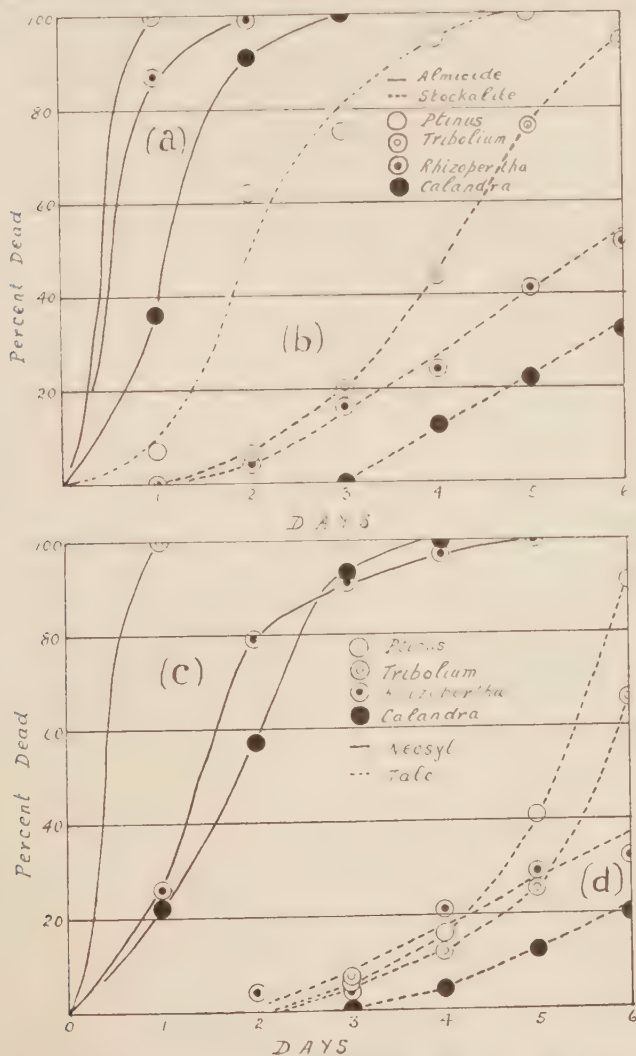


Fig. 21.—(a) Almicide, (b) Stockalite, (c) Neosyl, (d) Talc. The four species of beetles were not equally resistant to the desiccating action of the dusts, but the relative order of resistance did not change in different dusts. Tests were made at 25°C. and 60 per cent. R.H. Each point is based on three determinations by the continued contact method.

changes in the properties of the dusts on insecticidal action but the subject may also be considered from the standpoint of the insects. Two kinds of variation can be distinguished when considering the insects, namely the differences which occur between species and those which occur among the members of one species, and are associated with such factors as age, sex and nutrition. The tests described are mainly concerned with the former category of differences.

Age and sex in relation to dust action.

It was considered that a study of the influence of the age and sex of the test insects on their response to insecticidal dust action was not particularly relevant to the present investigation. In order to standardise these factors however the beetles were generally used for tests when they were about 14-28 days old and it was assumed that the sex ratio remained constant within the batches used for one experiment.

The species compared in relation to their response to dust action.

The levels of resistance of insects to the desiccating and toxic actions of dusts may vary from culture to culture and it should be remembered that these fluctuations, within a species, limit the accuracy and consistency of the results of comparative tests between species.

A batch of each species of beetle was taken when the insects were about 14-28 days old and their relative resistance to the desiccating action of several dusts was determined by the continued contact method at 25°C. and 60 per cent. R.H. It was found that the relative order of resistance remained the same in Alnicide, Neosyl, Stockalite and talc (figs. 21, 22).

It was noted that batches of insects varied in their resistance to the desiccating action of dusts. Since loss of water was responsible for the death of the insects it seemed probable that any condition which might lead to a higher moisture content in the beetles, such as a more humid environment and more moist food, would increase their resistance. In order to test this suggestion *Calandra granaria* 0-14 days old were transferred to batches of grain which had been conditioned at 32 per cent. R.H. and 76 per cent. R.H. for 14 days previously and were kept at these humidities for a further 14 days. They were then exposed to slate dust by the continued contact method at 25°C. and 60 per cent. R.H. It was found that the insects from the more humid environment were appreciably more resistant to desiccation (Table XXXII).

TABLE XXXII.

Exp. No.	Humidity of conditioning per cent. R.H.	Treatment given	Percentage killed after days			
			3	4	5	6
1	32	Slate dust	3	28	82	97
	76	Slate dust	2	10	50	81
	32	Control	0	0	0	8
	76	Control	0	0	0	0
2	32	Slate dust	4	49	90	100
	76	Slate dust	0	27	65	82
	32	Control	2	2	2	2
	76	Control	0	0	0	0

The relative order of resistance of the four species of beetles to the desiccating action of dusts is shown in fig. 21 a, b, c, d. Their resistance to the desiccating action of dry air was not the same and it was found that *Calandra* was the most rapidly killed under these conditions. These differences in the relative order of resistance to desiccation under the two circumstances could perhaps be explained by taking the activity of the beetles into account. *Calandra* was obviously more active than the other species and it lost weight more rapidly and succumbed more readily than the other insects under dry air conditions (Table XXXIII).

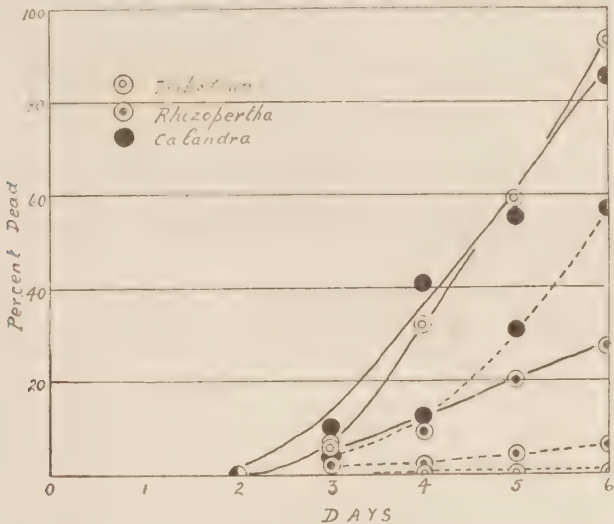


Fig. 22.—The four species of beetles differed in their resistance to DDT, the hairiest being the most resistant. When adherence came into play a smooth beetle appeared to be resistant to DDT because little dust adhered to it. *Ptinus* is not shown because it gave no kill. Tests conducted at 25°C. and 100 per cent. R.H. in triplicate using in all 60 insects for each determination. Continued contact=unbroken lines, discontinued contact=broken lines.

There were slight differences between the moisture contents of the various species but they could not be correlated with their resistance to desiccation either in dusts or in dry air. Duplicate determinations gave the following values for the moisture contents after drying for six hours at 105 C.—*Tribolium* 46.5 per cent. wt., *Calandra* 46.6 per cent. wt., *Rhizopertha* 52.2 per cent. wt. and *Ptinus* 45.4 per cent. wt.

TABLE XXXIII.

Time (hr.)	Percentage weight lost by			
	<i>Tribolium</i>	<i>Calandra</i>	<i>Rhizopertha</i>	<i>Ptinus</i>
6	3.1	3.6	4.3	2.6
24	5.9	9.5	8.1	5.8
48	10.2	18.1	13.3	10.5
71	15.1	25.3	17.3	15.0
120	25.7	43.0	25.2	23.2
168	37.0	—	33.6	31.5
Percentage dead at last weighing ...	34	100	16	18

Fifty beetles exposed to air at 0 per cent. R.H. at 25°C.

As would be expected very little dust adhered to insects which had smooth cuticles but the quantity increased rapidly as the insects became more ornamented with hairs and bristles. This effect was seen very clearly with the four beetles, *Tribolium*, *Calandra*, *Rhizopertha* and *Ptinus*, used in the present experiments which showed a progressive increase in hairiness. The dust which clings to *Tribolium* must do so largely by interfacial forces since there was little opportunity for the mechanical lodgement so abundantly provided by the hairs of *Rhizopertha* and *Ptinus*. The quantity adhering in each case after five minutes exposure using the discontinued contact method is shown for a variety of dusts in Table XXXIV, the data for which were obtained by weighing the beetles before and after dusting.

TABLE XXXIV.

Dust	Quantity of dust adhering							
	<i>Tribolium</i>		<i>Calandra</i>		<i>Rhizopertha</i>		<i>Ptinus</i>	
	µg./10	mg./g.	µg./10	mg./g.	µg./10	mg./g.	µg./10	mg./g.
Almicide	0	0.0	20	0.8	20	11.2	360	12.6
Kaolin	50	3.3	680	25.5	720	48.6	1,060	36.0
Slate	70	4.5	500	19.3	1,220	75.9	1,930	65.9
Talc	100	6.6	560	21.0	600	37.2	920	32.7
Silica	60	4.0	320	12.5	540	34.8	600	20.5

Determinations were made by weighing 50 or 100 insects. The results are given first as micrograms of dust adhering to 10 beetles then as mg. of dust per gram of beetles. Tested at 25°C. and 60 per cent. R.H.

Batches of the four beetles were left in continued contact with 5 per cent. DDT in kaolin at 100 per cent. R.H. As can be seen from fig. 22, *Tribolium* and *Calandra* were almost equally susceptible while the effect on *Rhizopertha* was much less and that on *Ptinus* negligible. It is interesting to note that the most hairy beetle *Ptinus* was most resistant to DDT, that *Rhizopertha* was both less hairy and less resistant, while *Tribolium* and *Calandra* were both much less hairy and also more susceptible. These observations suggested the possibility that the hairs acted as a barrier and kept the DDT away from the parts of the cuticle where it might otherwise have been absorbed.

TABLE XXXV.

Dust 10% w/w DDT on	Quantity of dust adhering							
	<i>Tribolium</i>		<i>Calandra</i>		<i>Rhizopertha</i>		<i>Ptinus</i>	
	µg./10	mg./g.	µg./10	mg./g.	µg./10	mg./g.	µg./10	mg./g.
Almicide	70	4.1	260	9.9	480	31.0	1,040	40.5
	60	3.5	300	11.4	480	31.2	1,280	49.8
Kaolin	120	7.0	360	13.7	800	52.0	1,480	57.5
	120	7.0	480	18.2	760	49.5	1,440	56.0
Slate	80	4.7	460	17.4	440	28.6	880	34.2
	100	5.8	400	15.1	360	23.5	800	31.2
Silica	80	4.7	320	12.1	520	33.8	800	31.1
	60	3.5	540	20.4	640	41.7	840	32.7

The results of two experiments are given first as micrograms of dust adhering to ten beetles and then as mg. of dust per gram of beetles.

When exposed to DDT dusts by the discontinued contact method at 100 per cent. R.H. giving an initial 5 mins. contact with excess of dust, *Ptinus* and *Rhizopertha*, which were resistant to DDT, were but little affected while the kill of *Tribolium* was (in contrast with the continued contact method) less than that of *Rhizopertha*. This apparent decline in the susceptibility of *Tribolium* occurred because practically no dust adhered to their smooth cuticles. In the case of *Calandra* rather more dust adhered and the beetles still showed an appreciable response. *Tribolium* showed large difference in the effects produced by the continued and discontinued contact procedures since in the latter method very little dust remained adhering. *Rhizopertha* and *Ptinus* retained the dust best but were resistant to its action, while *Calandra* occupied an intermediate category; it retained a moderate amount of dust and was fairly susceptible to its action (fig. 22 and Table XXXV).

Summary and Conclusions.

The methods used to investigate the properties of dusts are described and, in a theoretical section, the relevance of the various physical properties to insecticidal action are considered.

In order to investigate the effect of toxic and non-toxic dusts on insects the experimental procedure was simplified to eliminate all difficulties associated with the formation of uniform dust clouds and deposits. In problems relating to the adherence of the dusts to insects the actual quantity of dust was measured either by weighing the insects before and after dusting or by dyeing the dust with Sudan III and determining the quantity colorimetrically. The experiments were all conducted under known conditions of temperature and humidity.

Non-toxic dusts killed insects by causing them to lose water. Not all non-toxic powders were equally effective when conditioned to the same relative humidity. All were without effect at saturated humidity and became progressively more rapid in action as the humidity at which the test was carried out was decreased (p. 32).

The non-toxic dusts caused the insects to lose water by abrading certain areas of the cuticle; the more extensive the abrasion the more quickly the insects died (p. 27).

To be effective as an abrasive the dust must be hard and finely ground and, perhaps also, sharply angular. Thus materials which ranked high in Moh's scale of hardness were in general more effective than soft materials and hard materials became quite ineffective unless they contained material below about $10\ \mu$ diameter. Presumably the coarser materials could not gain access to the articulations, etc., where abrasion usually occurred. This effect can be seen clearly with carborundum powders. Sharply angular glass was more effective than the same powder converted into rounded spheres (pp. 25-31).

Visual inspection alone gave a very inaccurate assessment of the quantity of dust adhering to an insect. A voluminous dust of high specific surface always appeared to be much more adherent than a more dense dust of lower specific surface area. In comparing two dusts the true weights of material adhering were often found to be the reverse of those suggested by inspection.

A given material was more adherent when finely rather than coarsely powdered. This fact was well seen with different grades of Carborundum, Aloxite and slate. On the other hand, when different materials were compared, the most finely powdered, as determined by observations on the particle size in a dispersing medium or measurement of specific surface was not necessarily most adherent. The reason for this has not been determined, it may well be that under the circumstances of the dusting experiments the powders existed as aggregates (p. 25).

Insects eat the small particles of a powder more readily than the large particles and by using graded powders, such as "Carborundum", it was observed that the quantity in the gut became less as the particle size increased (pp. 27-28).

With the test insects used particles were seldom found in the spiracles, but when they were it was only the smallest (pp. 28-29).

No very marked effects attributable to particle shape were demonstrated. Almicide which was in the form of very thin flakes was, contrary to expectation, poorly adherent. A powder containing glass flakes was rather more abrasive than a powder consisting of spherical particles prepared from it by heat treatment. When the shapes of the particles in a powder were very irregular the bulk density was low since the particles did not pack closely together (pp. 29-30).

The most rapidly lethal dusts were those which caused the most rapid loss of weight. They also caused the highest percentage kill for the lowest average loss of weight. When individual insects were weighed however, it was found that the loss of weight at the time of death was much more nearly equal in the slowly and the rapidly acting dusts than was suggested by the average figures for fifty or one hundred insects (p. 32).

When an insect was dying of desiccation it did not appear to obtain water by oxidising food reserves since the rate of loss of dry weight was the same in dusted and undusted insects (p. 36).

The relative order of effectiveness of the dusts in bringing about desiccation remained essentially the same for the four species of test insects. Minor variations of order occurred between dusts which were almost equally effective (pp. 32-52).

The relative order of resistance to the desiccating action of the dusts of the four test insects examined was not the same as their relative order of resistance to dry air and could not be correlated with the differences in their moisture contents (p. 53).

Insects conditioned to high humidity (76 per cent. R.H.) were more resistant to the desiccating action of dusts than insects conditioned to low humidity (32 per cent. R.H.) (p. 52).

More dust adheres to rough coated insects than to smooth coated insects (p. 54).

The effect of adding DDT to a dust on the rate of its action on insects depends upon the abrasiveness of the dust and the humidity of the environment. At saturated humidity DDT increased the effectiveness of all non-toxic dusts since they themselves were entirely inactive. At moderate humidities adding 5 per cent. of DDT to the dusts increased the rate of action of the non-abrasive dusts, had little effect on the rather highly abrasive dusts Neosyl and reduced the rate of action of the most abrasive dust Almicide (pp. 42-43).

The most abrasive dusts, such as Almicide and Neosyl, killed insects more rapidly at 60 per cent. relative humidity than pure *p.p.*' DDT (p. 41).

Abrasive dusts were more effective carriers for DDT than non-abrasive dusts because the lethal effects of desiccation were added to those of the DDT (p. 38).

There was no evidence that abrasive dusts facilitated the entry of DDT or rotenone into insects as a result of the damage caused to the cuticle (p. 46).

There was a progressive increase in hairiness from *Tribolium* to *Calandra* to *Rhizopertha* to *Plinus* and a parallel increase in resistance to DDT dusts. This may suggest that the hairs formed barriers which kept the DDT from the sites where penetration usually occurred (p. 54).

After exposing beetles to an excess of dust and then vibrating them in a sieve, the quantity which remained adhering to a given species was found to be relatively constant for a particular dust and it was difficult to reduce the amount below this level by further vibration (pp. 22-23).

The quantity of DDT carrier dusts which remained adhering to insects varied for different carriers (pp. 45, 55).

Although very different quantities of the various DDT carrier dusts adhered to insects, they appeared to be almost equally toxic which, in view of the next conclusion can only mean that the DDT was not equally available in all cases (p. 45). Chemical estimations of the quantities of dusts adhering will therefore not measure their biological effectiveness.

In contrast with the conclusions just presented, if the quantity of any one carrier DDT dust adhering to insects was varied by sucking off some of the dust it was found that the kill was lowest in the case of insects carrying the least dust. The failure to detect differences in the case of the various carriers (as reported in the previous conclusion) cannot therefore be due to the method being inadequately sensitive (p. 45).

The dust adhering to insects was lost most slowly on the smoothest substratum (glass) more quickly on a rougher substratum (filter paper) and most quickly when foodstuffs were also present (pp. 23-24).

When dusts were compounded by adding the DDT to the carrier dusts in a solvent which was subsequently evaporated they were more toxic than those prepared by simply mixing the ingredients together (p. 48).

By the solvent application method the DDT may be assumed to be distributed over the surface of the carrier but there was no evidence that even an enormous increase in the specific surface area (which might be viewed as a dilution effect) led to a reduction in the toxicity of the dust (p. 48).

The addition of oil to a plain carrier or to a DDT carrier dust did not appreciably increase the toxic action. Oil did, however, increase the quantity of dust adhering to smooth-coated insects (but not to rough) and under those circumstances there was a considerable increase in toxic action (pp. 48-50).

The test insects were very resistant to rotenone and neither an abrasive carrier nor the presence of a mineral oil in the dust appreciably increased the toxic action of rotenone dusts (p. 50).

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References.

- ABBOTT, W. S. (1925). A method of computing the effectiveness of an insecticide.—*J. econ. Ent.*, **18**, pp. 265–267.
- ALEXANDER, P., KITCHENER, J. A. & BRISCOE, H. V. A. (1944a). Inert dust insecticides. I. Mechanism of action.—*Ann. appl. Biol.*, **31**, pp. 143–149.
- ALEXANDER, P., KITCHENER, J. A. & BRISCOE, H. V. A. (1944b). Inert dust insecticides. II. The nature of effective dusts.—*Ann. appl. Biol.*, **31**, pp. 150–156.
- ALEXANDER, P., KITCHENER, J. A. & BRISCOE, H. V. A. (1944c). Inert dust insecticides. III. The effect of dusts on stored products pests other than *Calandra granaria*.—*Ann. appl. Biol.*, **31**, pp. 156–159.
- AMER. SOC. TEST. MAT. (1941). Symposium on new methods for particle size determination in the subsieve range. 111 pp. Philadelphia, Pa.
- ANDERSEN, K. T. (1934). Biologie des Kornkäfers (*Calandra granaria* L.).—*Nachr. SchädlBekämpf.*, **9**, pp. 105–131.
- BACK, E. A. & COTTON, R. T. (1926). The Granary Weevil.—*Bull. U.S. Dep. Agric.*, no. 1393, 35 pp.
- BERTHOLF, L. M. & PHISON, J. E. (1941). Studies on toxicity to honeybees of acid lead arsenate, calcium arsenate, phenothiazine and cryolite.—*J. econ. Ent.*, **34**, pp. 24–33.
- BIRCH, L. C. (1945). The influence of temperature on the development of the different stages of *Calandra oryzae* L. and *Rhizopertha dominica* Fab.—*Aust. J. exp. Biol. med. Sci.*, **23**, pp. 29–35.
- BLISS, C. I. (1935). The calculation of the dosage-mortality curve.—*Ann. appl. Biol.*, **22**, pp. 134–167.
- BORCHERS, F. & MAY, E. (1935). Betrachtungen und Untersuchungen über die physikalischen Eigenschaften staubförmiger Pflanzenschutzmittel.—*Mitt. biol. Reichsanst.*, **50**, pp. 5–55.
- BUSVINE, J. R. & BARNES, S. (1947). Observations on mortality of insects exposed to dry insecticidal films.—*Bull. ent. Res.*, **38**, pp. 81–90.
- CAMPAU, E. J., WILSON, H. F. & JANES, R. L. (1942). Increased toxicity with rotenone dusts.—*Soap*, **18**, no. 8, pp. 100–101, 103.
- CHIU, S. F. (1939). Toxicity studies of so-called "inert" materials with the bean weevil *Acanthoscelides obtectus* (Say).—*J. econ. Ent.*, **32**, pp. 240–248.
- DALLA VALLE, J. M. (1943). Micromeritics—the technology of fine particles. 428 pp. London, Pitman.
- DAVID, W. A. L. (1946a). The quantity and distribution of spray collected by insects flying through insecticidal mists.—*Ann. appl. Biol.*, **33**, pp. 133–141.
- DAVID, W. A. L. (1946b). Factors influencing the interaction of insecticidal mists and flying insects. II. The production and behaviour of kerosene base insecticidal spray mists and their relation to flying insects.—*Bull. ent. Res.*, **37**, pp. 1–28.
- DEAN, E. W. & STARKE, O. D. (1920). A convenient method for the determination of water in petroleum and other organic emulsions.—*Industr. Engng Chem.*, **12**, pp. 486–490.
- EWER, D. W. & EWER, R. F. (1942). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae) a pest of stored products. III. The effect of temperature and humidity on oviposition, feeding and duration of life cycle.—*J. exp. Biol.*, **18**, pp. 290–305.

- FITZGIBBON, M. (1943). Seed disinfection. The determination of the adhesiveness of seed dressings to cereal seeds.—J. Soc. chem. Ind., **62**, pp. 8–11.
- GERMAR, B. (1936). Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln.—Z. angew. Ent., **22**, pp. 603–630.
- GOOD, N. E. (1936). The flour beetles of the genus *Tribolium*.—Tech. Bull. U.S. Dep. Agric., no. 498, 58 pp.
- GOODHUE, L. D. (1937). Particle size of commercial calcium arsenates by sedimentation analysis.—J. econ. Ent., **30**, pp. 466–474.
- GREGG, S. J. (1947). Absorption and heat of wetting methods of surface area measurements.—Trans. Instn chem. Engrs, Lond., **25**, Suppl. (Symp. Particle-size Anal.) pp. 40–46.
- GUNN, D. L. & KNIGHT, R. H. (1945). The biology and behaviour of *Ptinus tectus* Boie, a pest of stored products. VI. Culture conditions.—J. exp. Biol., **21**, pp. 132–143.
- HAMILTON, A. G. (1937). The mechanism of respiration of locusts and its bearing on the problem of the inhalation of poison dusts.—Bull. ent. Res., **28**, pp. 53–68.
- HEUBERGER, J. W. (1942). The tenacity of protective fungicides.—Chron. bot., **7**, pp. 9–10.
- HEUSCHMANN, O. (1929). Ueber die electrischen Eigenschaften des Insektenhaares.—Z. vergl. Physiol., **10**, pp. 594–664.
- HEWLETT, P. S. (1947). The toxicities of three petroleum oils to the grain weevils.—Ann. appl. Biol., **34**, pp. 575–585.
- HEYWOOD, H. (1938). Measurement of the fineness of powdered materials (and discussion).—Proc. Instn mech. Engrs, Lond., **140**, pp. 257–347.
- HEYWOOD, H. (1947). The scope of particle size analysis and standardisation.—Trans. Instn chem. Engrs, Lond., **25**, Suppl. (Symp. Particle-size Anal.) pp. 14–24.
- HICKIN, N. E. (1942). The food and water requirements of *Ptinus tectus* Boieldieu (Coleopt., Ptinidae).—Proc. R. ent. Soc. Lond., (A) **17**, pp. 99–108.
- HINTON, H. E. (1941). The Ptinidae of economic importance.—Bull. ent. Res., **31**, pp. 331–381.
- HOCKENYOS, G. L. (1933). Effect of dusts on the oriental roach.—J. econ. Ent., **26**, pp. 792–794.
- HODGMAN, C. D. (1945). Handbook of Chemistry and Physics. 29th edn. 2640 pp. Cleveland, Ohio, Chemical Rubber Publ. Co.
- HOWE, R. W. (1943). Life history data for *Ptinus tectus* Boie, at 70 per cent. relative humidity at 21°C. and 25°C.—Proc. R. ent. Soc. Lond., (A) **18**, pp. 63–65.
- HUNT, C. R. (1947). Toxicity of insecticide dust diluents and carriers to larvae of the Mexican Bean Beetle.—J. econ. Ent., **40**, pp. 215–219.
- LEE, F. M. & NURSE, R. W. (1939). The specific surface of fine powders.—J. Soc. chem. Ind., **58**, pp. 277–283.

- LEE, F. M. & NURSE, R. W. (1947). Permeability methods of fineness measurement.—Trans. Instn chem. Engrs, Lond., **25**, Suppl. (Symp. Particle-size Anal.) pp. 47-56.
- MCGOVYAN, E. R., CASSIL, C. C. & MAYER, E. C. (1940). Particle size of paris green as related to toxicity and repellency to the Mexican Bean Beetle.—J. econ. Ent., **33**, pp. 525-531.
- MCGREGOR, E. A. (1934). The relationship of fineness of sulphur particles to effectiveness against the citrus thrips in central California.—J. econ. Ent., **27**, pp. 543-546.
- MACLEOD, G. F. & SMITH, L. M. (1943). Deposits of insecticidal dusts and diluents on charged plates.—J. agric. Res., **66**, pp. 87-95.
- MOTE, D. C., WILCOX, J. & DAVIS, E. G. (1926). The natural "cleaning up" habit of insects.—J. econ. Ent., **19**, pp. 745-748.
- PARKIN, E. A. (1944). Control of the granary weevil with finely ground mineral dusts.—Ann. appl. Biol., **31**, pp. 84-88.
- POTTER, C. (1935). The biology and distribution of *Rhizopertha dominica* (Fab.).—Trans. R. ent. Soc. Lond., **83**, pp. 449-482.
- ROLLER, P. S. (1930). The bulking properties of microscopic particles.—Industr. Engng Chem., **22**, pp. 1206-1208.
- ROY, D. N. & GHOSH, S. M. (1944). The mechanism of action of a contact insecticide.—Bull. ent. Res., **35**, pp. 161-170.
- SHAFFER, G. D. (1915). How contact insecticides kill. III. Tech. Bull. Mich. agric. Exp. Sta., no. 21, 67 pp.
- SHIPITZINA, N. K. (1935). Grandeur maximum et minimum des particules pouvant être avalées par les larves d'*Anopheles maculipennis messeae*. [In Russian, with French summary].—Med. Parasit., **4**, pp. 381-389.
- SKINNER, D. G., BOAS-TRAUBE, S., BROWN, R. L. & HAWKSLEY, P. G. W. (1944). Method of determining particle size in sub-sieve range.—Brit. Coll. Owners Res. Ass. & Brit. Coal Util. Res. Ass., pp. 1-69. London.
- SMITH, C. L. (1936). The relation between the degree of fineness of pyrethrum powder produced by different periods of grinding to toxicity to insects and to deterioration by light and air.—J.N.Y. ent. Soc., **44**, pp. 317-339.
- SMITH, C. M. & GOODHUE, L. D. (1942). Particle size in relation to insecticide efficiency.—Industr. Engng Chem., **34**, pp. 490-493.
- STREETER, L. R. & RANKIN, W. H. (1930). The fineness of ground sulphur sold for dusting and spraying.—Tech. Bull. N.Y. St. agric. Exp. Sta., no. 160, 16 pp.
- VOELKEL, H. (1929). Die Bestimmung der Haftfähigkeit von Stäubemitteln.—Arb. biol. Reichsanst., **17**, pp. 253-272.
- WEBB, J. E. (1945a). The penetration of derris through the spiracles and cuticle of *Melophagus ovinus*, L.—Bull. ent. Res., **36**, pp. 15-22.
- WEBB, J. E. (1945b). On the respiratory mechanism of *Melophagus ovinus* L.—Proc. zool. Soc. Lond., **115**, pp. 218-250.

- WHITMAN, V. E. (1926). Studies in the electrification of dust clouds.—*Phys. Rev.*, **28**, pp. 1287–1301.
- WIGGLESWORTH, V. B. (1944). Action of inert dusts on insects.—*Nature, Lond.*, **153**, pp. 493–494.
- WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects.—*J. exp. Biol.*, **21**, pp. 97–114.
- WIGGLESWORTH, V. B. (1947). The site of action of inert dusts on certain beetles infesting stored products.—*Proc. R. ent. Soc. Lond.*, (A) **22**, pp. 65–69.
- WILCOX, J. (1926). A suggestion as to how phytophagous insects may ingest powdered poison.—*J. econ. Ent.*, **19**, 189–190.
- WILCOXON, F. & McALLAN, S. E. A. (1931). The fungicidal action of sulphur. III. Physical factors affecting the efficiency of dusts.—*Contr. Boyce Thompson Inst.*, **3**, pp. 509–528.
- WILSON, H. F. & CAMPAU, E. J. (1943). The effect of oil in rotenone dust mixtures.—*Soap*, **19**, no. 6, pp. 123, 125, 127.
- WILSON, H. F., JAMES, R. J. & CAMPAU, E. J. (1944). Electrostatic charge effects produced by insecticidal dusts.—*J. econ. Ent.*, **37**, pp. 651–655.
- WILSON, R. E. & FUWA, T. (1922). Humidity equilibria of various common substances.—*Industr. Engng Chem.*, **14**, pp. 913–918.
- ZACHER, F. (1937). Neue Untersuchungen über die Einwirkung oberflächenaktiver Pulver auf Insekten.—*Verh. dtsch. zool. Ges.*, pp. 264–271.
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OBSERVATIONS ON MOSQUITO BEHAVIOUR IN NATIVE HUTS.

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(Plate I.)

Mosquitos in an engorged condition have been observed leaving DDT-treated native huts. These may have picked up a lethal dose and died subsequently, or they may have made insufficient contact with the insecticide and survived.

Kennedy (1947) has shown in the laboratory that sub-lethal doses of DDT excite mosquitos and that they move preferentially to light when thus activated. As a result of this "contact-repellent" effect, mosquitos may become stimulated and leave native huts before acquiring a lethal dose. Hadaway and Barlow (1949) have shown that, when DDT in kerosene solution is applied to standard mud blocks, only some 6-15 per cent. of the DDT is present in the outer layer of the mud of 0.1 mm. thickness even at high dosages. Mosquitos may alight for some considerable time on a mud wall treated with a solution of DDT in kerosene and not obtain a lethal dose. Inadequate treatment may explain the survival of mosquitos leaving huts.

Observations were, therefore, made in specially constructed native huts to determine the effect of treatment with different insecticidal formulations on the entry and departure of mosquitos.

In Occupied, Untreated Huts.

Two circular huts were constructed, each with a mud wall and grass roof; they were 10 ft. in diameter with mud walls 6 ft. in height and $8\frac{1}{2}$ ft. to the apex of the roof. Five rectangular apertures with wooden frames 15 ins. long and 5 ins. wide were left in the walls approximately one foot from the top of the wall. Cotton sheeting curtains were fitted over the apertures so that each hut could be closed completely when required.

The huts were sited 100 yards from a brickfield where *Anopheles gambiae* Giles, was breeding, and about $\frac{1}{4}$ mile from a swamp on the edge of Lake Victoria. An African mosquito searcher and another African slept in each hut during the course of the experiments.

TABLE I.

Mosquitos caught in huts at two-hourly periods during the night: two huts on 11 nights between 17.vii.46 and 31.vii.46.

Time	Total		<i>A. gambiae</i>		Other Anophelines		<i>T. fuscopennatus</i>		<i>T. africanus</i>		Other Culicines	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
8 p.m.	6	1,360	4	41	0	4	0	1,211	0	13	2	91
10 p.m.	7	1,173	3	44	0	5	0	1,027	0	20	4	77
12 p.m.	6	819	1	62	0	5	0	674	3	19	2	59
2 a.m.	2	835	1	82	0	7	0	686	0	29	1	31
4 a.m.	1	867	0	99	0	4	1	682	0	29	0	53
6.30 a.m.	74	1,156	72	105	0	5	0	997	0	26	2	23

"Others" include specimens of *Taeniorhynchus aurites* (Theo.), *T. uniformis* (Theo.), *T. metallicus* (Theo.), *Culex poecilipes* Theo., *C. nebulosus* Theo., *C. tigripes* G. & C., and *Anopheles coustani* Lav.

Entry into huts.

The curtains were left open from just before dusk until 8 p.m., when they were dropped. Mosquitos in each hut were captured, as far as this was possible, with trained Africans using sucking tubes and these were transferred to small gauze cages for identification later. Catching operations usually lasted for 15 minutes. The curtains were then raised until 10 p.m. when the routine was repeated, and subsequently at two-hourly intervals throughout the night.

The captures made on 11 nights between 17.vii.46 and 31.vii.46 are shown in Table I. During this period the inside of the thatch roof was lined with mud-washed Amerikani sheeting to facilitate roof captures.

Mosquitos entered the huts at all times during the night, the peak periods being those at dusk from 6.30 until 8 p.m., and at dawn from 4 until 6.30 a.m. Specimens of *A. gambiae* were most numerous in the early morning captures, when there was a marked influx of males, presumably for shelter.

Morning-only captures compared with all-night captures.

Captures were made on 16 nights from 1.viii.46 to 23.viii.46, and the sheeting lining the roof was removed on 13.viii.46 to expose the thatch as in a normal native hut.

Each night two-hourly catches were made in one of the huts by the method previously described; in the other the apertures remained open throughout the night so that mosquitos could enter and leave at any time until the curtains were dropped at 6.30 a.m. for the morning-only catch. Morning-only captures were made in each hut on alternate days, consequently they were made in Hut 1 on eight of the days and in Hut 2 on the other eight.

The total numbers of mosquitos for the two huts are given in Table II. Those captured at 6.30 a.m. represented 40 per cent. of the total taken at two-hourly intervals throughout the night. Routine early morning captures in sample houses for the assessment of anti-malaria measures serve only as a rough index and do not give a true picture of the numbers of mosquitos entering and leaving these houses during the night.

TABLE II.

(a) Morning-only captures in Huts 1 and 2 on eight days.

Time	Total		<i>A. gambiae</i>		Other Anophelines		<i>T. fuscopennatus</i>		<i>T. africanus</i>		Other Culicines	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
6.30 a.m.	9	1,300	6	77	0	12	0	1,086	0	34	1	91

(b) Two-hourly captures in Huts 1 and 2 on eight days.

8 p.m.	1	758	1	18	0	7	0	648	0	22	0	63
10 p.m.	3	693	2	30	0	8	0	572	0	21	1	62
12 p.m.	2	474	0	86	0	4	1	314	0	27	1	43
2 a.m.	1	396	0	44	0	5	1	281	0	24	0	42
4 a.m.	0	431	0	46	0	1	0	330	0	19	0	35
6.30 a.m.	15	351	15	34	0	2	0	285	0	12	0	18
Total ...	22	3,103	18	258	0	27	2	2,430	0	125	2	263

"Others" include specimens of *Anopheles coustani*, *A. funestus* Giles, *A. marshalli* Theo., *Taeniorhynchus uniformis*, *T. metallicus*, *T. aurites*, *Culex poicilipes*, *Aedes lineatopennis* (Ludl.) and *A. africanus* Theo.

During this period, the total catches showed a peak of entry at dusk, but not at dawn, and the figures for *A. gambiae* showed a rise from dusk until 12 p.m. and then a gradual falling off to 6.30 a.m. Male *A. gambiae* again entered the huts at dawn.

The mean temperature and humidity inside Hut 1 at two-hourly intervals for the period 1.viii.46 to 23.viii.46 are given in Table III.

TABLE III.

Mean temperatures and humidities inside Hut 1 from 1.viii.46 to 23.viii.46.

Time	Temperature °F.	Relative Humidity %
8 p.m.	72.5	80.2
10 p.m.	71.9	81.4
12 p.m.	70.3	82.1
2 a.m.	69.4	84.1
4 a.m.	68.8	84.2
6.30 a.m.	68.8	84.4

Resting positions in huts.

Mosquitos in the two huts were segregated into those caught on the mud walls and those on the thatch roof. Out of a total of 5,576 caught, 63 per cent. were found on the roof and 79 per cent. of the 506 *Anopheles gambiae* taken were from the same position. Details are given in Table IV.

The resting positions of mosquitos are of importance when considering the application of insecticides to native huts and the relative toxicities of deposits on mud and grass thatch.

TABLE IV.

Resting positions of different species in huts.

Species	Total	No. on wall	No. on roof (% brackets)
<i>A. gambiae</i> ...	506	107	399 (79)
<i>A. coustani</i> ...	30	10	20 (67)
<i>A. marshalli</i> ...	9	4	5 (56)
<i>A. funestus</i> ...	7	2	5 (71)
<i>T. fuscopennatus</i> ...	4,475	1,822	2,935 (62)
<i>T. africanus</i> ...	217	110	107 (49)
<i>T. uniformis</i> ...	32	15	17 (53)
Totals ...	5,576	2,070	3,506 (63)

Departures from huts.

An attempt was made to determine the length of time mosquitos stay inside native huts. Traps of wire gauze on wooden frameworks were made to fit tightly into the five apertures in the wall. The one way opening into each trap was a horizontal slit 15 inches long and $\frac{1}{4}$ to $\frac{3}{8}$ inches wide, and mosquitos entering the traps could be removed when required through a cotton gauze sleeve on the outside.

The curtains were rolled up so that mosquitos could enter the hut from 7 p.m. until 10 p.m. The traps were placed in position at 10 p.m. so that no more mosquitos could enter the hut and those attempting to leave were caught in the traps. These were transferred from the traps to cotton gauze cages at two-hourly intervals, and at 6.30 a.m. those remaining in the hut were captured. Details of captures are given in Table V.

Of 1,014 female mosquitos entering the huts before 10 p.m., 63 per cent. were still inside, either on the roof or walls, at 6.30 a.m.; 71 per cent. of *A. gambiae* remained in the huts for this period.

TABLE V.

Departure of female mosquitos from Huts 1 and 2 on nine nights from 27.viii.46 to 6.ix.46 and on eight nights from 11.xi.46 to 22.xi.46.

	Total	<i>A. gambiae</i>	<i>T. fuscopennatus</i>	<i>T. africanus</i>	Others
In traps from					
10-12 p.m....	117	19	75	14	9
12- 2 p.m....	81	11	48	10	12
2- 4 p.m....	83	5	60	13	5
4-6.30 p.m....	104	7	60	21	16
In huts at 6.30 a.m. ...	629	101	387	64	77
Totals ...	1,014	143	630	122	119

Captures were made in one hut on 16 nights from 18.ii.47 to 14.iii.47 to determine the numbers of mosquitos attempting to leave at different times during the night. Mosquitos could enter the hut from 7 p.m. to 9 p.m., when the traps were placed in position. The mosquitos in the hut were caught at 11 p.m., and those that had attempted to leave between 9 p.m. and 11 p.m. were transferred from the traps to small gauze cages.

Similarly mosquitos were allowed to enter the hut from 11 p.m. to 1 a.m., and from 3 a.m. to 5 a.m., and captures in the hut and traps were made at 3 a.m. and 7 a.m. Details are given in Table VI.

Of 753 mosquitos caught, 28 per cent. were in the traps. This means that 72 per cent. of the mosquitos entering the hut from 7 to 9 p.m., 11 to 1 a.m., and 3 to 5 a.m. remained in the hut for at least two hours. Of the 59 *A. gambiae* captured, 44 (74 per cent.) remained in the hut for at least two hours.

TABLE VI.

Departures of mosquitos from Hut 2 on 16 nights from 18.ii.47 to 14.iii.47.

	Total	<i>A. gambiae</i>	Other Anophelines	<i>T. fuscopennatus</i>	<i>T. africanus</i>	Other Culicines
11 p.m. Hut ...	139	17	5	89	21	7
Traps ...	56	2	3	35	10	6
3 a.m. Hut ...	160	11	3	108	29	9
Traps ...	51	2	2	37	2	8
7 a.m. Hut ...	240	16	9	168	36	11
Traps ...	107	11	6	57	23	10

"Others" include *Anopheles funestus*, *A. coustani*, *Taeniorhynchus metallicus*, *T. uniformis*, *Culex nebulosus* and *C. poicilipes*.

In Occupied, Treated Huts.

Three circular huts similar to those described previously, and at the same place, were used for these experiments. Huts 3 and 4 were ten yards apart, and Hut 2 (of the previous experiments) was a further 30 yards from Hut 3. The bait consisted, as before, of one sleeper and one mosquito searcher in each hut.

Hut No. 4 was treated on 5.v.47 with a water suspension of a wettable powder, Neocid B.A. 50, manufactured by Geigy Co., New York. The suspension, containing 5 per cent. DDT (pp') w/v, was applied by means of a Four Oaks "Kent" Sprayer fitted with one nozzle, of $\frac{1}{16}$ th inch diameter aperture, and pumped to a pressure of 50 lb. per square inch. Three pints of the insecticide were applied to the inside of the walls and roof, an area of approximately 300 sq. ft., i.e. a dosage of 285 mg. DDT (pp') per sq. ft.

Dosages determined from sample papers four ins. square varied considerably, despite every effort to achieve an even coverage. Three papers on the wall had dosages of 544, 227 and 204 mg. DDT (pp') per sq. ft. and two papers on the roof, 771 and 114 mg. per sq. ft.

The five apertures in the wall were covered and protected during the spraying so that the traps would not become contaminated when in use.

It was originally intended to treat Hut 3 at the same time as Hut 4, and to keep Hut 2 as an untreated control, but unfortunately the latter partially collapsed. Hut 3 was therefore kept as a control for Hut 4 until Hut 2 was repaired, and it was not treated until 30.vi.47. The insecticide used was a 5 per cent. DDT (pp') solution made by dissolving commercial DDT, containing 81 per cent. pp' isomer, in power kerosene. Three pints of the solution were applied as described above. Five sample papers on the wall had dosages of 74, 74, 58, 58 and 93 mg. DDT (pp') per sq. ft., and three on the roof, 280, 74 and 58 mg. per sq. ft.

Entry of Mosquitos.

Catches were made in the huts on five nights during the first week after the application to Hut 4. The apertures were opened and mosquitos allowed to enter from 7 p.m. to 9 p.m. The curtains were then lowered and mosquitos in the hut were caught in sucking tubes and transferred to cotton gauze cages. The curtains were again raised, and the procedure repeated at two-hourly intervals until 7 a.m. Two-hourly catches during the night were made subsequently on five nights of alternate weeks.

The gauze cages from each hut were kept in a wooden box, covered with moist lint, and were returned to the laboratory in the morning. Twelve hours after the time of capture the mosquitos were identified and recorded as dead or alive. The cotton gauze covers and wire frames of the cages were washed each day in hot soap and water, and were selected at random for subsequent use so that any possibility of a build-up of a toxic deposit on the cages for any particular hut was avoided.

Observations showed that mosquitos entering Hut 4 after treatment with DDT wettable powder were as numerous as before and the treatment did not interfere in any way with the normal behaviour as far as entry into the hut was concerned. Large numbers of mosquitos entered the hut after 7 p.m. when the curtains were opened, but by 8 p.m. there were considerably fewer present. Specimens in the hut became restless after making contact with treated surfaces; there were numerous short flights from one part of the roof or wall to another, and some mosquitos undoubtedly flew from the hut. Entries were still occurring between 7 p.m. and 9 p.m., of course, and accounted for the maintenance of the mosquito population at a diminished level. The mosquitos still alive in the hut were captured at 9 p.m.

New entries occurred when the curtains were again raised and the cycle was repeated. Peak periods of entry occurred at dusk and dawn, as in the untreated hut.

Dead mosquitos were seen to fall on a white sheet on the floor, but it was not possible to assess their numbers because of the occupation of the hut by the sleeper and searcher. Three weeks before the treatment of Hut 4, 743 female mosquitos were caught in Hut 4 and 442 in Hut 3. The numbers in the untreated Hut 3 rose to 533 one week after treatment of Hut 4, and to 1,005 seven weeks after. If the pre-treatment ratio had been maintained, 896 and 1,689 mosquitos would have been expected at these times in Hut 4; instead, the actual figures were 228 and 444 respectively. It would appear, therefore, that considerable numbers of mosquitos had either been killed in Hut 4 during the two-hour periods, or had been activated and had escaped from the hut before the curtains were lowered.

Three weeks before the treatment of Hut 4, 89 per cent. of 743 mosquitos were alive 12 hours after capture in Hut 4, and 88 per cent. of 442 in Hut 3. Only 2 per cent. of 228 mosquitos survived in Hut 4 during the first week after treatment, compared with 97 per cent. of 533 in the untreated Hut 3. The percentage survival in Hut 4 had risen to 33 during the 15th week after treatment. It is interesting to note that during the 3rd week, when a large untreated board bearing meteorological instruments was present in the hut, the survival rate was 16 per cent. The weekly total captures of mosquitos remaining alive in the huts at the end of each two-hour period, and their survival rates are given in Table VII. Details for some species are given in Table VIII. Survival rates are, of course, of mosquitos which had been in the hut for a maximum period of two hours.

Similar observations were made in Hut 3 after it had been treated with a 5 per cent. DDT solution in power kerosene. The number of mosquitos in the hut was large at the beginning of each two-hour period but decreased towards the end of that time. Survival rates of mosquitos remaining in the hut at the end of each two-hour period were higher than those in Hut 4. For example, five weeks after

TABLE VII.

Total mosquito captures made at two-hourly intervals from 9 p.m. to 7 a.m. on five nights each week.

Week from treatment		Hut 4		Hut 3		Hut 2	
of Hut 4	of Hut 3	Total	% alive	Total	% alive	Total	% alive
-3		743	89	442	88		
1		228	2	533	97		
3		482	16	556	98		
5	-4	438	1	1,088	98		
7	-2	444	6	1,005	93	402	98
9	1	334	20	182	26	276	92
11	3	no catch		259	76	429	97
13	5	438	34	285	56	320	95
15	7	433	33	346	59	427	98

Survivals recorded 12 hours after capture.

Hut 4 treated with water suspension of DDT wettable powder.

Hut 3 treated with solution of DD in kerosene.

TABLE VIII.

Captures of some species made at two-hourly intervals from 9 p.m. to 7 a.m. on five nights each week.

Species	Week from treatment		Hut 4		Hut 3		Hut 2	
	of Hut 4	of Hut 3	Total	% alive	Total	% alive	Total	% alive
<i>A. gambiae</i> ...	-3		20	100	13	100		
	1		0	—	8	100		
	3		8	50	6	100		
	5	-4	1	0	9	100		
	7	-2	2	50	5	100	1	100
	9	1	2	0	1	100	2	100
	11	3	no catch		0	—	2	100
	13	5	1	0	0	—	0	—
	15	7	0	—	0	—	0	—
<i>A. coustani</i> ...	-3		30	90	22	100		
	1		5	0	6	100		
	3		33	42	39	100		
	5	-4	15	0	36	100		
	7	-2	5	20	5	100	3	100
	9	1	1	100	1	0	2	100
	11	3	no catch		4	100	7	100
	13	5	4	100	1	100	1	100
	15	7	9	44	1	100	4	100
<i>T. fuscopennatus</i> ...	-3		539	88	338	86		
	1		175	3	450	96		
	3		338	12	387	98		
	5	-4	376	1	987	98		
	7	-2	420	5	947	93	379	97
	9	1	322	19	168	26	264	92
	11	3	no catch		240	75	400	97
	13	5	398	31	263	56	294	95
	15	7	382	31	314	58	377	97

Hut 4 was treated with DDT wettable powder on 3.v.47, Hut 3 with DDT solution in kerosene on 30.vi.47 whilst Hut 2 was untreated.

treatment the survival rate was 56 per cent. Deposits on the mud walls and thatch roof from the application of a water suspension of a DDT wettable powder were more toxic to mosquitos than the deposits from a DDT solution in kerosene.

Treatment of Huts 3 and 4 did not prevent mosquitos entering and feeding on the occupants. Bites were frequent at the beginning of a two-hour period, and occurred less frequently later in the period, but it is not possible to determine from the data whether feeding occurred after the acquisition of a lethal dose.

Temperatures and humidities recorded in one of the huts are given in Table IX.

TABLE IX.

(a) Mean temperatures °F. recorded in hut.

Week from Treatment of Hut 4	7 p.m.	9 p.m.	11 p.m.	1 a.m.	3 a.m.	5 a.m.	7 a.m.
-3 ...	71	71	70	69	69	68	68
1 ...	76	76	73	73	72	70	69
3 ...	73	72	71	70	70	69	69
5 ...	74	72	72	71	70	70	70
7 ...	75	76	76	72	72	71	69
9 ...	74	75	74	72	71	70	70
11 ...	75	75	73	72	71	71	69
13 ...	73	73	72	71	71	70	70
15 ...	72	73	73	72	71	70	69

(b) Mean humidities recorded in hut.

Week from Treatment of Hut 4	7 p.m.	9 p.m.	11 p.m.	1 a.m.	3 a.m.	5 a.m.	7 a.m.
-3 ...	86	86	84	81	79	77	77
1 ...	81	77	85	83	82	84	84
3 ...	84	84	86	86	87	86	84
5 ...	88	89	89	87	89	90	87
7 ...	76	74	72	77	79	75	83
9 ...	85	82	86	85	88	86	83
11 ...	88	88	85	86	89	89	86
13 ...	87	87	86	86	87	85	85
15 ...	87	85	85	86	88	82	82

Departure of mosquitos from huts.

Observations were made in the huts on five nights of alternate weeks commencing during the second week after the treatment of Hut 4 in an attempt to secure a comparison between the numbers of mosquitos leaving a treated and an untreated hut.

Traps of wire gauze on wooden frameworks were made to fit tightly into the five apertures in the wall of each hut. The entrance into a Mark I trap consisted of a horizontal slit 15 inches long by $\frac{1}{4}$ to $\frac{3}{8}$ in. wide, and five such slits were the only exits from the hut. This was considered to be too small, and Mark II traps were constructed so that each exit from the hut measured 15 by 5 ins. for the depth of the wall, and led to a trap through a horizontal slit as before. Mark II traps were used from 9.vi.47 onwards, but did not appear to affect the results to any great extent. Mosquitos usually find their way in and out of native huts of this type through small openings between the top of the walls and the thatch roof; windows and doors are kept shut after dark.

The curtains were open from 7 p.m. to 8 p.m., so that mosquitos could enter the hut freely. At 8 p.m. the traps were inserted in the apertures so that no more

mosquitos could enter the hut, and any attempting to leave were caught in the traps. At 9 p.m. all mosquitos in each hut were captured and placed in cotton gauze cages, and those in the traps were transferred to separate cages. Specimens caught in the hut at 9 p.m., therefore, had been in the hut for at least one hour, and not more than two hours. Catches were repeated at 11 p.m., 5 a.m. and 7 a.m., after mosquitos had been allowed to enter from 9 to 10 p.m., 3 to 4 a.m., and 5 to 6 a.m., and the traps had been in position from 10 to 11 p.m., 4 to 5 a.m., and 6 to 7 a.m. No observations were made between 11 p.m. and 3 a.m. when the hut was closed completely. Survival rates were recorded 12 hours after the time of capture.

Thus, mosquitos were allowed to enter and leave the hut for a period of one hour. At the end of that time the traps were placed in position. During the next hour no more mosquitos could enter the hut and any attempting to leave were caught in the traps. The percentage of the total catch in the traps at the end of this hour remained approximately the same before and after treatment of Huts 3 and 4. A higher percentage in the traps would have been expected after treatment if contact with DDT resulted in activation and flight from the hut. Kennedy (1947) found in laboratory tests that *Anopheles maculipennis atroparvus* van Thiel and *Aedes aegypti* (L.), activated by sub-lethal doses of DDT, moved preferentially towards light. During the course of these experiments a hurricane lamp had to be used in each hut.

Treatment of Hut 4 with DDT wettable powder appeared to be more effective than that of Hut 3 with DDT solution in kerosene. The survival rates given are for mosquitos which had been in the huts for more than one hour and less than two hours. None of the mosquitos from Hut 4 were alive 12 hours after capture during

TABLE X.

Total mosquito captures made at 9 p.m., 11 p.m., 5 a.m., and 7 a.m. on five nights each week.

Week from treatment		Hut 4				Hut 3			Hut 2		
of Hut 4	of Hut 3	Total	% alive	% of Total in traps	Total	% alive	% of Total in traps	Total	% alive	% of Total in traps	
-2		Hut Traps	683 93	93 93	12	632 202	96 96	24			
		Treated									
2		Hut Traps	170 15	0 0	8	419 191	98 96	31			
6	-3	Hut Traps	517 80	10 39	13	1,596 240	90 98	13			
8	-1	Hut Traps	306 25	8 24	8	618 197	83 94	24	316 64	91 100	
		Treated									
10	2	Huts Traps	446 70	11 31	14	259 85	79 78	25	181 58	97 93	
										24	

Windows open 7-8, 9-10, 3-4 and 5-6 a.m.

Traps in position 8-9, 10-11, 4-5 and 6-7 a.m.

Survivals recorded 12 hours after capture.

the second week after treatment, whereas survival rates from the traps and Hut 3 were 78 per cent. and 79 per cent. respectively. Ten weeks after treatment of Hut 4, the survival rate for the hut was 11 per cent., and for the traps 31 per cent.

Details of total captures and survival rates are given in Tables X and XI.

TABLE XI.

Captures of some species made at 9 p.m., 11 p.m., 5 a.m. and 7 a.m. on five nights each week.

Species	Week from treatment		Catches in	Hut 4		Hut 3		Hut 2	
	of Hut 4	of Hut 3		Total	% alive	Total	% alive	Total	% alive
<i>A. gambiae</i> ...	-2		Hut ...	1	100	1	100		
			Traps	2	100	1	100		
	2		Hut ...	0	—	6	100		
			Traps	0	—	3	100		
	6	-3	Hut ...	2	0	3	100		
			Traps	0	—	1	100		
	8	-1	Hut ...	2	50	9	100	6	100
			Traps	0	—	1	100		
	10	2	Hut ...	1	0	0	—	1	100
			Traps	0	—	0	—		
<i>A. coustani</i> ...	-2		Hut ...	34	100	12	100		
			Traps	5	100	5	100		
	2		Hut ...	8	0	22	100		
			Traps	1	0	14	100		
	6	-3	Hut ...	8	0	13	100		
			Traps	1	0	3	100		
	8	-1	Hut ...	5	0	4	100	6	100
			Traps	0	—	2	100		
	10	2	Hut ...	24	21	2	100	2	100
			Traps	2	50	0	—		
<i>T. fuscopennatus</i> ...	-2		Hut ...	599	92	547	95		
			Traps	77	91	173	95		
	2		Hut ...	138	0	334	97		
			Traps	14	0	148	95		
	6	-3	Hut ...	486	8	1,523	91		
			Traps	73	37	221	98		
	8	-1	Hut ...	288	8	550	83	294	91
			Traps	25	24	162	95		
	10	2	Hut ...	406	10	232	78	171	96
			Traps	61	33	82	78		

Hut 4 was treated with DDT dispersible powder on 5.v.47, Hut 3 with DDT solution in kerosene on 30.vi.47, whilst Hut 2 was untreated.

In Unoccupied, Treated Huts.

Experimental huts similar to those described previously were erected near the laboratory at Entebbe. They were empty and unoccupied, and were kept locked except when experiments were carried out. No mosquitos could enter the huts, and the only exits were the openings into the traps.

Female *A. gambiae* that had fed, were collected in native houses in the early morning, transferred to Barraud boxes and transported to the laboratory. They were moved individually at 4 p.m. into 2×1 inch glass tubes with cotton wool plugs, and at 8 p.m., after dark, about 50 mosquitos were released individually from the tubes into each hut. This method was found to be more accurate than storage of batches in cages and release direct from these. Mosquitos in the traps were recovered by means of sucking tubes at 9 p.m., 10 p.m., 7 a.m. and 8 a.m., and were placed in large recovery cages in the laboratory. Numbers alive were recorded 18 hours later. At 8 a.m. mosquitos still alive in the hut were recovered on white sheets by spraying with a pyrethrum-kerosene extract.

During the preliminary work, 373 female *A. gambiae* were released in the huts; the total recovery was 78 per cent., of which 12 per cent. were taken in the traps and 66 per cent. by flit-spraying. Only 1 per cent. of those recovered from the traps died within 18 hours. Mosquitos not accounted for may easily have been caught in the grass of the roof or cracks in the mud walls during the flit spraying, and not fallen on the sheets. In addition, there may have been a natural mortality rate. A sufficient and approximately constant recovery, however, was obtained by this method; the main disadvantage was that survivals of mosquitos recovered by flit spraying could not be determined.

Application of DDT in Huts 5 and 6.

At 8 a.m. on 3.iii.47 the interior of Hut 6 was sprayed with 5 per cent. DDT (pp') solution in power kerosene made by dissolving commercial DDT containing 81 per cent. para-para isomer. Application was made with a Four Oaks "Kent" Sprayer fitted with one nozzle held approximately one foot from the surface to be treated. A fairly even coverage was obtained over the walls, roof and central supporting pole. The wooden frames for the traps were carefully protected by covering the apertures, so that the traps themselves would not become contaminated when in position. Approximately 300 sq. ft. were treated with 2.4 pints to give an estimated dosage of 220 mg. DDT per sq. ft. The dosage on five sample papers, each four inches square, was 53, 87, 86 mg. pp' DDT per sq. ft. on the walls and 180, 54 mg. on the roof.

The interior of Hut 5 was sprayed with a water suspension, containing 5 per cent. DDT (pp'), of a wettable powder Neocid B.A. 50, manufactured by Geigy Co., New York.

The procedure was identical with that for Hut 6 but in this case the dosages on the five sample papers were 51, 88, 150 mg. pp' DDT per sq. ft. on the walls and 63, 130 mg. on the roof.

Hut 7 remained untreated.

Observations on mosquitos released for 12 hours in DDT-treated huts.

Releases of fed, female *A. gambiae* were made at intervals over a period of 17 weeks from the time of application of the insecticide. Details of recaptures are given in Tables XII, XIII and XIV.

The total numbers of *A. gambiae* released over the whole period after treatment were 319 in Hut 7 (untreated), 316 in Hut 5 (DDT wettable powder) and 338 in Hut 6 (DDT-kerosene solution), and the numbers taken from the traps one hour after their release were 18, 46 and 59 respectively. There was a tendency for more

mosquitos to enter the traps of the treated huts than those of the untreated one, but the figures are insufficient to show a significant result. The survival rate of mosquitos in the traps of Hut 7 was 95 per cent. ; of Hut 5, 85 per cent. ; and of Hut 6, 80 per cent. It would appear, therefore, that the majority of the mosquitos leaving the treated huts did so before acquiring a lethal dose.

Mosquitos were taken from the traps of treated huts later than one hour after the time of release only on one occasion. This was four weeks after the application of DDT-kerosene solution, when two were in the traps two hours after their release. No mosquitos were recovered by flit spraying 12 hours after the time of release in the hut treated with DDT wettable powder, and on two occasions only, single specimens were recovered from the hut treated with DDT-kerosene solution. Recoveries in the untreated hut, however, remained fairly constant. The balance of the unrecovered mosquitos in the treated huts, therefore, must have been killed by the insecticidal deposits during the 12-hour period after their release.

Meteorological conditions, recorded on the nights of experiments in a screen close to the huts averaged :—

Temperature at 9 p.m. 74°F. ; at 2 a.m. 71°F. ; at 8 a.m. 70°F.

Humidity at 8 p.m. 83 per cent. ; at 2 a.m. 88 per cent. ; at 8 a.m. 86 per cent. Releases, 4, 8 and 12 weeks after the application of insecticides were made when the moon was almost full, and light shone into the otherwise darkened huts through the wire gauze of the traps.

TABLE XII.

Hut 7.—Untreated.

Recoveries of fed female *A. gambiae* released into hut at 8 p.m.

Time from applications in treated huts	No. of mosquitos released	No. in Traps at				No. recovered by flitting at 8 a.m.	% Total Recovery
		9 p.m.	10 p.m.	7 a.m.	8 a.m.		
<i>Before</i>							
3 weeks	60	0	0	0	2 (2)	45	78
1 week	53	8 (8)*	3 (3)	0	2 (2)	33	87
<i>After</i>							
12 hours	53	5 (5)	1 (1)	0	1 (1)	36	81
1 week	44	1 (1)	0	3 (3)	1 (1)	28	75
2 weeks	50	2 (2)	0	0	0	35	74
4 weeks	47	5 (4)	0	4 (4)	0	26	75
8 weeks	46	3 (3)	2 (2)	5 (5)	0	22	70
12 weeks	48	2 (2)	1 (1)	2 (1)	0	29	71
17 weeks	31	0	0	1 (1)	0	18	61

*Numbers alive 18 hours later are shown in brackets.

Observations on mosquitos released for periods of from 1 to 6 hours in DDT-treated huts.

A. gambiae were released in the huts at 12 noon during the 23rd–26th weeks after treatment, and recoveries were made by flitting 1, 3 or 6 hours later. Traps were not used, the apertures being covered so that no mosquitos could leave the huts. Figures are given in Table XV. Fewer recoveries were made in the hut treated with wettable powder than in the one treated with a kerosene solution. Six hours after release, recoveries were only 2 per cent. and 7 per cent. respectively. The remainder had been knocked down or killed before the sheets were placed in position and the flit sprayings took place. The mosquitos recovered by flitting represented those still alive in the huts at that time ; it is not possible to say whether they would have survived if they had been removed from the huts and kept in untreated cages.

TABLE XIII.

Hut 5.—Treated with DDT wettable powder.
Recoveries of fed female *A. gambiae* released into hut at 8 p.m.

Time from application of insecticide	No. of mosquitos released	No. in Traps at				No. recovered by flitting at 8 a.m.	% Total Recovery
		9 p.m.	10 p.m.	7 a.m.	8 a.m.		
<i>Before</i>							
3 weeks	60	1 (1)*	1 (1)	0	1 (1)	45	80
1 week	53	3 (3)	2 (2)	0	3 (3)	35	81
<i>After</i>							
12 hours	53	21 (20)	0	0	0	0	40
1 week	45	1 (1)	0	0	0	0	2
2 weeks	51	6 (5)	0	0	0	0	12
4 weeks	46	9 (8)	0	0	0	0	20
8 weeks	51	8 (4)	0	0	0	0	16
12 weeks	36	1 (1)	0	0	0	0	3
17 weeks	34	0	0	0	0	0	0

*Numbers alive 18 hours later are shown in brackets.

TABLE XIV.

Hut 6.—Treated with 5 per cent. DDT solution in kerosene.
Recoveries of fed female *A. gambiae* released into hut at 8 p.m.

Time from application of insecticide	No. of mosquitos released	No. in Traps at				No. recovered by flitting at 8 a.m.	% Total Recovery
		9 p.m.	10 p.m.	7 a.m.	8 a.m.		
<i>Before</i>							
3 weeks	90	10 (9)*	4 (4)	0	3 (3)	46	70
1 week	57	0	0	0	3 (3)	45	78
<i>After</i>							
12 hours	63	6 (5)	0	0	0	0	10
1 week	64	8 (5)	0	0	0	1	14
2 weeks	49	6 (4)	0	0	0	0	12
4 weeks	53	26 (22)	2 (2)	0	0	1	55
8 weeks	40	5 (4)	0	0	0	0	12
12 weeks	39	7 (6)	0	0	0	0	18
17 weeks	30	1 (1)	0	0	0	0	3

*Numbers alive 18 hours later are shown in brackets.

TABLE XV.

Recoveries of *A. gambiae* by "flitting" at different times after their release in huts.

Weeks after application of insecticide	Exposure time in hut	% Recovery of Mosquitos		
		Hut 7, untreated	Hut 5, wettable powder	Hut 6, kerosene solution
23	1 hour	65 (48)	45 (33)	53 (51)
24	3 hours	84 (43)	10 (42)	19 (43)
26	6 hours	72 (51)	2 (57)	7 (57)

Numbers of mosquitos used are given in brackets.

Application of Benzene Hexachloride in Hut 8.

Hut 8 was treated with a water suspension of "Gammexane" wettable powder D.P. 530, containing 0.5 per cent. gamma isomer w/v, at 8 a.m. on 9.vi.47. The procedure was as described for the treatment of Huts 5 and 6. Three hundred square feet were treated with 3 pints of the insecticide to give an estimated dosage of 220 mg. benzene hexachloride per sq. ft. or 28.5 gm. gamma isomer per sq. ft.

The dosage on ten sample papers each four ins. square was 63, 79, 87, 94, 102 and 114 mg. benzene hexachloride per sq. ft. on the walls and 63, 71, 75, 87 mg. on the roof.

Observations on mosquitos released for 12 hours in BHC-treated hut.

Releases of female *A. gambiae* were made at intervals over a period of 12 weeks from the time of application of the insecticide. Details of recoveries are given in Table XVI.

Forty-six mosquitos were released at 8 p.m. in the hut 12 hours after treatment. Two dead specimens were found in the traps one hour later, but no further recoveries were made either in the traps or in the hut by flit spraying at 8 a.m. the following morning. None of the mosquitos released into the hut 1, 4 and 6 weeks after treatment escaped into the traps, nor were any alive in the hut 12 hours after their release. Thirty-five *A. gambiae* were released 12 weeks after treatment; three live specimens were in the traps at 9 p.m. and a further two at 10 p.m., but none was alive in the hut at 8 a.m. the following morning. Recoveries in the untreated hut remained fairly constant.

TABLE XVI.
Recoveries of fed female *A. gambiae* released at 8 p.m.

Time from application of insecticide	No. of mosquitos released	No. in Traps at				No. recovered by flitting at 8 a.m.	% Total Recovery
		9 p.m.	10 p.m.	7 a.m.	8 a.m.		
Hut 8.—Treated with Gammexane wettable powder							
<i>Before</i>							
2 weeks	44	2 (2)	0	2 (2)	0	26	68
1 week	38	3 (3)	1 (1)	2 (2)	0	20	68
<i>After</i>							
12 hours	46	2 (0)	0	0	0	0	4
1 week	37	0	0	0	0	0	0
4 weeks	50	0	0	0	0	0	0
6 weeks	45	0	0	0	0	0	0
12 weeks	35	3 (3)	2 (2)	0	0	0	14
Hut 7.—Untreated							
<i>Before</i>							
1 week	48	2 (2)	1 (1)	2 (1)	0	29	71
<i>After</i>							
12 hours	43	2 (2)	0	1 (1)	0	28	72
1 week	25	0	1 (1)	2 (2)	0	13	64
4 weeks	31	0	0	1 (1)	0	19	64
6 weeks	30	0	0	0	0	21	70
12 weeks	34	2 (2)	1 (1)	2 (2)	0	19	71

Numbers alive 18 hours later are shown in brackets.

It must be assumed, therefore, that except for the few escaping into the traps, all mosquitos had been killed during the 12-hour period after their release into the treated hut. From time to time, attempts were made to confirm this by spreading white sheets on the floor of the hut just before the mosquitos were released at 8 p.m.; and by inspecting them for dead specimens an hour later. This was done, for instance, when 46 *A. gambiae* were released 12 hours after the treatment of the hut; 25 dead specimens were found on the sheets one hour later. This procedure was not carried out regularly because inspection involved entering the hut, and if it was delayed until the following morning it was found that ants and spiders had removed a number of the dead mosquitos.

Observations on mosquitos released for periods of 1, 3 and 6 hours in BHC-treated hut.

During the 9th-11th weeks after treatment, *A. gambiae* were released in Hut 4 at 12 noon, and recoveries were made by flitting 1, 3 or 6 hours later. Recaptures, given in Table XVII, were higher than those in Huts 5 and 6, 23-26 weeks after treatment with DDT solution and wettable powder preparations.

TABLE XVII.

Recoveries of *A. gambiae* by "flitting" at different times after their release.

Weeks after application of insecticide	Exposure time in hut	% Recovery of Mosquitos	
		Hut 7, untreated	Hut 8, Gammexane wettable powder
9	1 hour	65 (48)	66 (41)
10	3 hours	84 (43)	43 (47)
11	6 hours	66 (45)	12 (42)

Numbers of mosquitos used are given in brackets.

Summary.

Mosquitos continue to enter occupied, untreated native huts throughout the night, with peak periods of entry at dusk and dawn. Early morning mosquito catches do not give a true picture of the numbers entering and leaving huts during the night.

In a series of catches 63 per cent. of 5,576 mosquitos and 79 per cent. of 506 *Anopheles gambiae* were caught resting on the underside of the thatch roof.

By using five traps inserted in apertures one foot below the top of the wall, the numbers of mosquitos attempting to leave a hut were determined. Of 1,014 mosquitos entering huts before 10 p.m., 63 per cent. remained inside until 6.30 a.m., that is for 8½ hours. Catches to estimate numbers entering and leaving at different times during the night were also made.

Treatment of huts with DDT wettable powder and DDT-kerosene solution did not interfere with the normal behaviour of mosquitos as far as entry was concerned. Biting occurred in the treated huts.

The DDT wettable powder appeared to be more effective than the DDT-kerosene solution.

Some mosquitos entered the treated huts, fed and then left before acquiring a lethal dose. After making contact with treated surfaces mosquitos became restless but, under the conditions existing in the huts during the experiments, activation did not result in more leaving the treated huts than the untreated one. Unfortunately there were few *A. gambiae* and the predominant species entering the huts was

Taeniorhynchus fuscopennatus.

Some of the female *A. gambiae* released into unoccupied DDT-treated huts escaped into the traps before acquiring a lethal dose. Although there was a tendency for more to enter the traps of a DDT-treated hut than those of an untreated hut, the data are insufficient to show a significant difference.

The majority of mosquitos entering the traps did so within one hour of their release.

No mosquitos were still alive 12 hours after their release in huts treated 17 weeks previously with DDT wettable powder or DDT-kerosene solution, or in the hut treated 12 weeks previously with "Gammexane" wettable powder.

References.

- HADAWAY, A. B. & BARLOW, F. (1949). Bull. ent. Res., **40**, pp. 323-343.
KENNEDY, J. S. (1947). Bull. ent. Res., **37**, pp. 593-607.
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FIG. 1. Experimental Hut with trap in position.



FIG. 2. Mark I traps.



FIG. 3. Mark II traps.



THE BIOLOGY OF *CEPHALONOMIA WATERSTONI* GAHAN (HYM., BETHYLIDAE), A PARASITE OF *LAEMOPHLOEUS* (COL., CUCUJIDAE).

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Cephalonomia waterstoni was described by Gahan (1931). Only the female had been found and the host was then unknown. Richards (1939) described the male and redescribed the female in a review of the British BETHYLIDAE. A record of a species of *Cephalonomia* by Durrant (1921) proved on later examination of his material to refer to *C. waterstoni* (Richards, 1939).

The earliest record of the host of *C. waterstoni* is in a paper by Schread and Garman (1933). *Laemophloeus minutus* (Oliv.) (Coleoptera, Cucujidae) was recorded as a pest of *Trichogramma* cultures and *C. waterstoni* as a parasite that kept the beetle in check. Schread and Garman stated that *C. waterstoni* stings *L. minutus* larvae, upon which it then feeds, finally burying them. Sheppard (1936) recorded *C. waterstoni* as a parasite of *Cryptolestes* (*Laemophloeus*) *ferrugineus* (Steph.) again in *Trichogramma* cultures and stated that eggs as well as larvae of *L. ferrugineus* are fed upon by the adult parasite.

TABLE I.
Stored products pests and their Bethyid parasites.

Host	Bethyid	Author
<i>Laemophloeus ferrugineus</i> (Steph.)	<i>Cephalonomia waterstoni</i> Gahan	Sheppard (1936).
<i>L. minutus</i> Oliv.	{ <i>C. waterstoni</i> <i>Plastanoxus westwoodi</i> (Kieffer) }	Schread and Garman (1933). Gahan (1931).
<i>(L. pusillus</i> Schøn.)		
* <i>L. turcicus</i> Grouv.	<i>C. waterstoni</i>	Finlayson (1950).
* <i>L. sp. indet.</i>	<i>C. waterstoni</i>	Finlayson (1950).
<i>Oryzaephilus surinamensis</i> (L.)	<i>C. tarsalis</i> (Ashm.)	Gahan (1931).
	<i>(C. carinata</i> (Kieff.)	Richards and Herford (1930).
	<i>(C. sp. indet.)</i>	Myers (1929).
	<i>C. meridionalis</i>	Brèthes (1913).
	<i>C. hyalinipennis</i> Ashm.	Ashmead (1893)R, Bridwell (1919).
	<i>C. destructrix</i> Guss.	Gussakovskii (1935).
<i>Tribolium castaneum</i> (Herbst)	? <i>C. tarsalis</i>	Richards (1939).
<i>T. confusum</i> Duval	<i>Rhabdepyris zeae</i> Turner & Waterston	Waterston (1921).
<i>Calandra oryzae</i> (L.)	<i>C. tarsalis</i>	Fouts (1920)R.
	<i>(C. kiefferi</i> Fouts)	
<i>Sitodrepa paniceum</i> (L.) ...	<i>C. gallicola</i> Ashm.	van Emden (1931). van Emden (1931). van Emden (1931). *Bridwell (1919). *van Emden (1931). Kearns (1934).
	<i>(C. quadridentata</i> Duch.) ...	
* <i>Ptinus tectus</i> Boield.	<i>C. gallicola</i>	
* <i>Niptus hololeucus</i> Fald. ...	<i>C. gallicola</i>	
<i>Lasioderma serricorne</i> F. ...	<i>C. gallicola</i>	
<i>Tenebroides mauritanicus</i> L.	<i>C. nigricornis</i> Sarra	Sarra (1930).
<i>Ephestria elutella</i> (Hb.) ...	<i>Holepyris hawaiiensis</i> (Ashm.)	Bridwell (1920)R.
<i>Plodia interpunctella</i> (Hb.) ...	<i>H. hawaiiensis</i>	Bridwell (1920)R.
?	<i>Plastanoxus munroi</i> Richards	Richards (1939).

*—Cases where parasitisation occurred in laboratory experiments.
R—Reference taken from Richards (1939) ; original paper not seen.

A description by Myers (1929) of the general behaviour of a species of *Cephalonomia* parasitising *Oryzaephilus surinamensis* (L.), applies equally well to *C. waterstoni*. According to Gahan (1931), some specimens of *C. waterstoni* may have been among Myers' material. The species of *Cephalonomia* studied by Myers was not identified by him but Gahan considered that it was probably *C. tarsalis* (Ashm.). Larvae of *Oryzaephilus surinamensis* and *O. mercator* (Fauv.) have been supplied to *C. waterstoni* by the present author, but no eggs were ever laid although a few larvae were stung and paralysed. It seems unlikely, therefore, that Myers had any *C. waterstoni* in his stock. A more detailed account of the biology of *C. gallicola* (Ashm.) is given by van Emden (1931) and short accounts of *C. gallicola* and *C. tarsalis* are given by Kearns (1934) and Powell (1938).

Several insect pests of stored products are known to be attacked by Bethyids and the list given in Table I is probably fairly exhaustive.

Field Work.

The stock of *C. waterstoni* used in the studies recorded in this paper was obtained at Sharpness, Glos., in a shed of Manitoba wheat which was heavily infested with *Laemophloeus minutus* and *L. ferrugineus*. The latter were identified according to the key devised by Reid (1942). Other insects were present in small numbers, chiefly *Oryzaephilus surinamensis*, *Calandra oryzae* and *Ahasverus advena* Waltl, but it was obvious that only the *Laemophloeus* population was large enough to account for the numbers of *Cephalonomia* present. The *Laemophloeus* population was concentrated in two "hot-spots" like the one described by Oxley and Howe (1944); *Cephalonomia* was present in large numbers in both.

The insect population and the moisture content of the grain were determined from samples taken at various depths by means of a large sampling spear described by Lucas and Glover (1946) and a simpler model described by Oxley and Henderson (1944). Temperatures were taken with long thermocouple ropes, inserted by temporary attachment to metal rods screwed end to end, and a potentiometer as described by Oxley and Henderson (1944).

Both "hot-spots" were extensive and at an advanced stage in development; central temperatures being well over 40°C.

It is interesting to compare the numbers of insects found with the figures given by Oxley and Howe (1944) and Lucas and Oxley (1946) for other *Laemophloeus* "hot-spots" in wheat. In the present case the numbers of *Laemophloeus* are very much lower than those given by Oxley and Howe for an infestation at about the same stage in development, *i.e.*, with maximum temperatures above 40°C. Their figures include "wandering" (extragranular) larvae but in the present case only three of the samples contained extragranular larvae and the numbers are so small that they do not affect the validity of the comparison. Table II gives the numbers of *Laemophloeus*

TABLE II.

Oxley and Howe		Finlayson	
Temperature	Numbers of <i>Laemophloeus</i> per kg.		Temperature
* approx. 18°C.	265·6	12	18·9°C.
30°C.	2,855	48	30°C.
37·5°C.	298-1,444	152	37·3°C.
		120	38·8°C.
41°C.	132·8	40·5	42·3°C.

* Not given by Oxley & Howe—estimated by present author.

per kilogram for a few approximately corresponding temperatures in the two infestations. Below 20°C. very few living *Laemophloeus* were found in the present infestation but in the one described by Oxley and Howe fair numbers were present. It seems probable that *Cephalonomia* had considerably reduced the numbers of *Laemophloeus* and had slowed up the spread of the infestation into the cooler regions of the grain bulk. The numbers found at all points below 20°C. were too small to cause heating without secondary, local aggregations or local differences in humidity or temperature arising from factors other than the original "hot-spot" (Oxley & Howe, 1944).

The figures given by Oxley and Howe do not include larvae which were living inside grains because at that time it was believed that *Laemophloeus* was free-living in all its stages. In the account of another infestation given later by Lucas and Oxley (1946) the numbers of intragranular larvae were determined by breeding out for six weeks in the laboratory. This infestation had not reached the maximum temperatures which occur in heating grain, viz., 40–44°C., the highest temperature recorded being below 36°C. The adult *Laemophloeus* population (an unidentified species to be mentioned later) between 25° and 30° C. was in the region of 100–300 per kilogram. In the present case about 50 per kilogram were found at these temperatures. As migration occurs from regions of very high temperature (30–36°C., Lucas & Oxley, 1946) and probably also from regions of dense population it is difficult to draw a valid comparison between the Lucas and Oxley infestation and the present one. The larval population in the Lucas and Oxley account, however, reached very high figures, e.g., over 3,000 per kilogram at 34–36°C. and over 1,000 at 27°C. The larval population in the present case, as shown by breeding out at 25°C. and 70 per cent. R.H. for seven weeks, reached very high numbers in only one region around 37.3°C. from which 3,780 adults per kilogram were bred. At all other temperatures, the numbers bred out could have been accounted for by oviposition by the adults present in the samples during the three days before they were sieved off. The samples were left for one week too long before the final sieving and most of the adults bred out were obtained during this final week. Even if all of the adults bred out came from larvae present at the time of collection, the numbers are far below those found by Lucas and Oxley for similar temperatures, e.g. Lucas and Oxley, 30°C.—about 2,000 larvae; present author 30°C.—maximum of 146 larvae; Lucas and Oxley, 34°C.—over 2,000 larvae; present author, 33.4°C.—maximum of 507 larvae.

Cephalonomia probably migrates to regions of very high temperature; the largest numbers were found at 38.8°C., 42.3°C. and 43.1°C. (omitting the surface samples). It does not follow that these very high temperatures are near the optimum for development, but it seems likely that *Cephalonomia* can develop at higher temperatures than *Laemophloeus*. At 42.3°C., for instance, 553 *Cephalonomia* were bred out, but only 189 *Laemophloeus*, all of which had probably developed from eggs laid during the interval between collection and examination, since (judging from observations made in the laboratory) the maximum temperature for the development of *Laemophloeus* is under 40°C. The host larvae on which the 553 *Cephalonomia* had developed must have been several weeks old at the time of parasitisation and at that time the local temperatures would be lower.

Near the surface, the populations of *Laemophloeus* and *Cephalonomia* were very large showing that both tend to migrate either upwards, or away from the very high temperatures immediately below. *Laemophloeus* tends to move upwards regardless of the grain temperature. Aggregations of *Laemophloeus* at the surface of silo bins of wheat and oats have been observed by the present author where the temperature of the whole bulk was very uniform and not greater than 18°C. at any point. The total population of *Laemophloeus* was extremely small and there was no possibility of the aggregations being due to migration from regions of dense population, such as may occur in a "hot-spot".

About 400 living *Cephalonomia* from a surface sample were sexed by Dr. O. W. Richards and the author, and all were found to be females. Later studies have shown that the sex ratio is about 2 : 1♂ in laboratory cultures but the males live for only a few days, which possibly explains this result.

Bethylids have been observed to cause irritation to man by stinging (e.g. van Emden, 1931). This was confirmed for *C. waterstoni* in the field work by Mr. J. D. Jones of the Pest Infestation Laboratory, D.S.I.R., and the author. The irritation, which was greatest on the neck, may have been caused, however, by the release of secretions from crushed insects. When crushed, *Cephalonomia* produces a very characteristic, pungent odour which was very strong in the shed of wheat at Sharpness. This odour is obvious even when a single specimen is crushed between the fingers.

Culture Methods.

Various culture methods were tried, using at first *Laemophloeus minutus* as it is easier to breed in large numbers at 25°C. and 70 per cent. relative humidity than *L. ferrugineus* which does better at a higher temperature. *Cephalonomia* females were placed in cultures of *L. minutus* on flour, rolled oats and yeast containing all stages of the beetle. Very few parasites were produced by this method. *L. ferrugineus* and *L. minutus* larvae at all stages were then supplied in large numbers and the stocks were increased on *L. ferrugineus* but not on *L. minutus*. Accordingly, for later cultures *L. ferrugineus* was used as host. Apart from being more acceptable, *L. ferrugineus* has another advantage over *L. minutus* in that it does not produce silk until it starts to form its cocoon. *L. minutus* (also *L. turcicus*) produces silk while still moving about and consequently the culture medium becomes rather matted if there are many larvae in a small amount of food. At present *Cephalonomia*, for use in host selection experiments, is reared on *L. ferrugineus*, *L. minutus* and *L. sp. indet.* (Lucas & Oxley, 1946). *L. turcicus* produces too few parasites under the conditions used to be of any practical value as a culture host. A satisfactory culture procedure for *Cephalonomia* using 2×1 in. glass tubes, is as follows:—

Ten adult *L. ferrugineus* are placed in a food mixture consisting of equal parts rolled oats and bran, with a pinch of dried brewer's yeast or wheat germ.* The 2×1 in. tubes are half to threequarters filled with the food mixture and kept at 30°C. and 80 per cent. relative humidity for three weeks. The *Laemophloeus* larvae are then about one week from pupation and almost fully grown. The adult *Laemophloeus* are removed and six female and one or more male *Cephalonomia* added. The next generation of *Cephalonomia* adults begins to emerge after a fortnight.

Cephalonomia can squeeze through extremely narrow crevices and frequently escapes from tubes stopped with a perforated cork covered with muslin. It was found advantageous to seal the tubes with a strip of adhesive cellophane tape bound tightly round the junction of stopper and tube. This was found more convenient than paraffin wax.

Cultures were made up weekly as above and a constant supply of parasites obtained. *Cephalonomia* lives for only a few days without food and it was found easier to sub-culture weekly rather than attempt to keep stocks of adults alive for long periods by feeding them with sugar solution.

General Survey of Life-cycle and Behaviour.

After emergence from the cocoon, the female wasp readily attacks the first suitable *Laemophloeus* larva encountered and equally readily accepts the first male that attempts to mate with her. No cases have been seen of males entering

* *L. ferrugineus* cannot be sexed satisfactorily without dissection but in cultures using the other species as hosts, five females only need be added.

cocoons to mate with females before they have emerged as occurs in some Bethyids, e.g. *C. gallicola* (van Emden, 1931). Copulation is a brief procedure which lasts for a few minutes and which apparently occurs only once in the lifetime of each female. No cases of repeated copulation were observed when males were introduced into tubes containing females which were known to have copulated previously.

When a female encounters a larva, she palpates it for a short time with her antennae, then seizes it at any spot, and attempts to sting it. No particular site is selected for the insertion of the sting. The *Laemophloeus* larva has been observed to escape quite frequently, especially in the case of *L. ferrugineus* whose larva is more active than those of the other three species studied. This greater activity of *L. ferrugineus* is also very noticeable in the adult. If, as is usual, the *Cephalonomia* female succeeds in stinging the larva, paralysis occurs within a few seconds. When the sting is inserted at the end of the body stiffening begins at that end and spreads gradually until the whole larva has stopped wriggling. Even after successful stinging, a larva will twitch occasionally and move its legs in an unco-ordinated manner. No recovery from stinging, as occurs in the hosts of several species of *Goniozus* (e.g., *G. claripennis* Foerst., Voukassovitch, 1924), has been observed in *Laemophloeus*.

The paralysed larva is carried aloft or dragged into a sheltered position, e.g., under a flake of bran. Sometimes the female wasp feeds on the larva before carrying it off. A small hole is bitten in the integument and some of the body fluids imbibed. Very often the larva is discarded without any eggs being laid on it although it may be returned to later, whether by chance or "design" is not yet known. If oviposition takes place, usually one or two, rarely three and very rarely four eggs are laid attached to the host. Eggs laid by virgin females produce only males (arrhenotoky) as is usual in the Bethyids. Keeler (1929a, b) claimed that virgins of *Scleroderma immigrans* Bridwell produce only female offspring (thelytoky) but Bridwell (1929) in studies on the same species found that virgins produced male offspring. Clausen (1940) put forward the view that there may be two biological races of this species, one arrhenotokous and the other thelytokous.

The egg soon hatches but the exact moment of hatching is difficult to detect because there is little difference in gross appearance between the egg and the early larva. The criterion used to make this distinction is the insertion of the head of the parasite into the host, which produces an anterior curving of the larva. Sometimes it is very difficult to decide if hatching has occurred without moving the egg or larva slightly to see if it is really attached at one end. This is a dangerous procedure as the egg or early larva is easily dislodged. Powell (1938) described hatching in *C. tarsalis* and stated that the head of the larva emerged from the chorion of the egg and was inserted into the host.

The larval stage lasts for only a few days during which growth is very rapid. A number of white urate cells in the fat body are very conspicuous, showing through the transparent body wall. Sometimes fully grown larvae are pink in colour. This pink coloration has been noted in various Bethyids, and in *Goniozus claripennis* disappears gradually from the anterior end and vanishes entirely with the ejection of the meconium prior to pupation (Voukassovitch, 1924). The meconium in *G. claripennis*, as in *C. waterstoni*, is dark red to black in colour. This pink substance may be an excretory product but it is by no means universal in *C. waterstoni* larvae, probably less than 25 per cent. showing it.

When the *Cephalonomia* larva (or larvae) has finished feeding, only a shrivelled vestige of the host larva remains. *Cephalonomia* then begins to spin, moving its anterior region from side to side in wide sweeps to anchor threads and a tough, dense, ovoid, white cocoon is formed in a suitable situation among food particles or debris.

If a *Cephalonomia* larva is left on a flat surface, a proper cocoon cannot be formed and only a tangled mass of threads is produced. Male larvae tend to pupate without forming a proper cocoon even in a favourable situation. The meconium is extruded by the prepupa inside the cocoon and oozes through, often anchoring the cocoon to the substrate at the posterior end. Powell (1938) stated that the meconium of *C. gallicola*, as it dries, forces the head of the pupa up against the wall of the cocoon. When an individual pupates without forming a cocoon, it may be elevated to an angle of 60°. In the cocoon, the maximum angle which can be formed is 30° and so Powell concluded that the pupa must be under considerable tension. In cases where the meconium was broken and the pupa was allowed to lie flat, Powell found that development was halted or slowed down considerably. If the pupa was made to roll about, however, no such retardation occurred. Powell stated that a "damp layer" formed below the pupa if it was allowed to remain stationary and he thought that this "damp layer" had something to do with the change in the rate of development.

The prepupal stage lasts for two or three days at 25°C. The pupal stage, which is the longest one in the pre-imaginal life of *C. waterstoni*, lasts for about a fortnight.

Experimental Work.

In all of the experiments described below, unless stated to the contrary, *L. ferrugineus* was used as host.

Life-history.

In order to determine the duration of the various stages in the life-cycle, experiments were carried out at constant temperatures of 25 and 30°C. and relative humidities of 60 and 80 per cent. The humidity control was effected by means of KOH solutions adjusted to give the correct humidity at 20°C.; no allowance was made for the effect of other temperatures on the humidity. The theoretical variation in relative humidity produced by these solutions between 20 and 30°C. is very slight and probably less than the actual variations from the desired relative humidity which occur under experimental conditions.

Eggs were obtained by enclosing female *Cephalonomia* with *Laemophloeus ferrugineus* larvae and removing parasitised larvae daily. The *Laemophloeus* larvae and the egg-laying wasps were kept at the same conditions of temperature and humidity as those in which the eggs were subsequently reared in the experiments. The parasitised host larvae were placed in small tubes and examined daily. Each combination of the two temperatures and two relative humidities were used in these experiments.

As stated in the general survey of the life-cycle it is difficult to tell a newly hatched larva from an egg but it was attempted and the period from oviposition to hatching is given in the results. No attempt was made to count ecdyses. No exuviae were seen but no special effort was made to detect them. The prepupal and pupal stages spent inside the opaque cocoon cannot be observed without opening the cocoon. The stages most easily noted and quite satisfactory for comparative purposes are as follows:—

Egg laid	...	eclosion
Eclosion	...	cocoon fully formed
Cocoon	...	emergence of adult

The results are shown in Table III. Statistical analysis of the results was carried out according to Mather (1946). Sheppard's correction factor was not applied. The means for the total life cycle of males and females combined were compared and the values for $P\left(c=\frac{d}{sd}\right)$ are shown in Table III.

TABLE III.
Duration of stages in the life-cycle of *Cephalonomia waterstoni*.

Period		25°C. and 60 per cent. R.H.			30°C. and 60 per cent. R.H.		
		Mean No. of days and S.E.	No. of insects	Mean range in days	Mean No. of days and S.E.	No. of insects	Mean range in days
Egg laid-eclosion	...	2.21	48	0.5-2.5	1.53	64	0.5-2.5
(Egg laid cocoon)	...	(6.23)	47	(0.5-5.5)	(3.92)	61	(0.5-3.5)
Eclosion-cocoon	...	4.02	47	2.5-5.5	2.39	61	1.5-3.5
Cocoon emergence	...	15.13	18	13.5-16.5	10.53	58	8.5-11.5
Total life-cycle ♂	...	20.91 ± 0.18	21	19.5-21.5	14.59 ± 0.18	22	12.5-15.5
P	...	<0.04			>0.88		
Total life-cycle ♀	...	21.54 ± 0.22	24	19.5-22.5	14.62 ± 0.11	42	12.5-15.5
" (♂ + ♀)	...	21.25 ± 0.15	45	19.5-22.5	14.61 ± 0.09	64	12.5-15.5
		P(♂ + ♀) <0.94	M.11.9 per cent.	S.D. 9.5 mm.	P(♂ + ♀) >0.56	M.9 per cent.	S.D. 12.7 mm.
		25°C. and 80 per cent. R.H.			30°C. and 80 per cent. R.H.		
Egg laid-eclosion	...	2.04	28	0.5-2.5	1.09	45	0.5-1.5
(Egg laid-cocoon)	...	(5.74)	31	(0.5-4.5)	(4.12)	34	(0.5-3.5)
Eclosion cocoon	...	3.90	19	2.5-4.5	3.05	49	1.5-3.5
Cocoon-emergence	...	15.64	22	13.5-16.5	10.78	36	8.5-11.5
Total life-cycle ♂	...	21.33 ± 0.33	9	20.5-21.5	14.64 ± 0.34	11	13.5-15.5
P	...	>0.47			>0.82		
Total life-cycle ♀	...	21.59 ± 0.14	22	19.5-22.5	14.78 ± 0.32	27	13.5-15.5
" (♂ + ♀)	...	21.41 ± 0.20	34	19.5-22.5	14.73 ± 0.20	45	13.5-15.5
			M.36.5 per cent.	S.D. 5.0 mm.		M.20 per cent.	S.D. 6.4 mm.

There is no significant difference between the means for the total life-cycle at 60 per cent. R.H. and 80 per cent. R.H. and either 25°C. or 30°C. There is a highly significant difference between the means for the total life-cycle at 25°C. and 30°C.

The means for the total life-cycle of males and females calculated separately were also compared and no significant difference found except possibly at 25°C. and 60 per cent. R.H. ($P = < 0.04$).

The following conclusions may be drawn from the results :—

(i) At all of the experimental conditions, the egg stage and the feeding larval stages are very short, taking less than a third of the time spent by the prepupal stages within the cocoon.

(ii) A difference of 20 per cent. relative humidity (between 60 and 80 per cent.) at 25 or 30°C. has no effect on the duration of development. ($P > 0.94$ at 25°C. ; $P > 0.56$ at 30°C.)

(iii) The total period from oviposition to the emergence of the adult from the cocoon is about 3 weeks at 25°C. and 2 weeks at 30°C.

(iv) There is no statistically significant difference between the means for total periods of development of males and females except possibly at 25°C. and 60 per cent. R.H.

(v) The mortality rate (M) at both humidities was lower at 30°C. than at 25°C. Also, at both temperatures it was lower at 60 per cent. relative humidity than at 80 per cent. Calculation of the saturation deficits (S.D.) at each combination of temperature and relative humidity shows that mortality increased with decrease in saturation deficit (Table III). (See p. 93 on parthenogenesis where similar figures were obtained for 25°C., 30°C., and 80 per cent. relative humidity.)

The duration of all stages is similar to those found in *C. gallicola* by van Emden (1931). Myers (1929) stated that *C. tarsalis* took 31 days for development but he did not record any temperatures. In *C. gallicola* (van Emden, 1931 ; Kearns, 1934) and in *C. tarsalis* (Powell, 1938) the male develops more rapidly and emerges from the cocoon before the female. Except at 25°C. and 60 per cent. R.H. there is no significant difference between males and females in *C. waterstoni* at the temperatures used in the experiments but reference to Table III will show that in all four experiments the mean period of development of the males is slightly less than that of the females. This may become a significant difference at lower temperatures.

Longevity of adults without food.

The results shown in Table IV were obtained in two ways. The first set (A) was obtained by recording the duration of adult life of males and females which were reared in the life-history experiments and which had, therefore, been reared at the same conditions as those in which they were kept in the longevity experiments. The second set (B) was obtained by isolating cocoons from cultures which had been kept at 30°C. and 80 per cent. relative humidity and keeping the adults which emerged at the four sets of conditions used in the experiments. In both A and B examinations were made daily. In A the humidity was controlled by KOH solutions only but in B a solution at H_2SO_4 was used for 60 per cent. relative humidity and KOH for 80 per cent.

Experiment B was carried out to supplement experiment A because the number of insects used in the latter was rather small. However, the results in the two cases are different and have accordingly been given separately. In experiment A the adults lived for a longer period at 80 per cent. relative humidity at each temperature than at 60 per cent. relative humidity ; the expected result. In experiment B the opposite is the case ; the adults live for a longer time at 60 per cent. relative humidity at each temperature. Another difference between the results of the two

experiments is the greater range at most conditions in experiment B. No satisfactory explanation of these differences can be offered, but as the total numbers of insects are small no great significance can be attached to them.

TABLE IV.

Longevity in days of males and females—without food.

Conditions		Males			Females		
		No.	Mean longevity	Mean range	No.	Mean longevity	Mean range
30°C./80 per cent. R.H.	A	13	4.77	2.5-6.5	24	4.04	1.5-6.5
	B	8	2.50	0.5-4.5	16	2.88	0.5-4.5
30°C./60 per cent. R.H.	A	11	3.18	1.5-5.5	19	3.68	1.5-6.5
	B	13	3.31	1.5-5.5	27	3.07	1.5-4.5
25°C./80 per cent. R.H.	A	3	5.38	4.5-6.5	12	5.66	3.5-7.5
	B	13	4.69	0.5-8.5	23	4.04	0.5-6.5
25°C./60 per cent. R.H.	A	9	4.55	2.5-6.5	15	4.60	2.5-6.5
	B	16	5.38	0.5-9.5	10	4.20	1.5-7.5

Longevity of adults with host larvae available.

During many periods of observation, in which *Cephalonomia* females were watched attacking and feeding upon *Laemophloeus* larvae, males in the same containers were never seen feeding or showing any interest in the blood exuding from wounded larvae. In order to test the hypothesis, based on these observations, that males do not feed on host larvae, the following experiment was set up:—

Three series of six (2 in. × 1 in.) tubes were set up—

Series A containing bran only.

„ B „ „ +50 normal *L. ferrugineus* larvae.

„ C „ „ +50 paralysed *L. ferrugineus* larvae.

Series C was set up to test the hypothesis that males feed on larvae only after they have been paralysed by females.

Males were obtained from single female cultures (see p. 82). Cocoons taken from these cultures were examined daily and males removed to the experiment when they emerged. At the beginning of the experiment each male was, therefore, less than one day old. Corresponding tubes in each series were supplied with three males which were the offspring of the same female. The experiment was carried out at 30°C. and 80 per cent. relative humidity.

The results in the three series gave a longevity in days for males with host larvae available in Series A varying from 2 to 5 days with a mean of 3.8 days, in Series B from 3 to 6 with a mean of 4.5 days and in Series C from 2-6 with a mean of 3.8 days.

After the six males in series B were dead, 50 unharmed host larvae were recovered from each tube showing that the males had not attacked them. No significant difference in longevity was recorded in the three series and it may be concluded that males do not feed on *Laemophloeus* larvae, whether normal or paralysed.

To determine the length of life of females when fed with host larvae, two experiments were set up, one at 25°C. and 80 per cent. relative humidity and one at 30°C. and 80 per cent. relative humidity. At 25°C., one female (0-1 day old) was placed in each of four tubes (2 in. \times 1 in.) containing 50 *L. ferrugineus* larvae on a food mixture consisting of rolled oats, bran, dried brewer's yeast and wheat germ; at 30°C. eight similar tubes were set up. Additional batches of 10 larvae were added from time to time when most of the experimental ones were paralysed. Examinations were made at intervals of 1-4 days. At 30°C., four tubes were discarded before the females were dead because eggs had been overlooked in the examinations and daughter females had emerged. As the larval stage is so short and the cocoon is usually covered with food particles, it is very easy to overlook a few offspring unless daily examinations are carried out.

TABLE V.
Longevity and fecundity of females on *L. ferrugineus* larvae.

Conditions	♀	Longevity in days	No. of eggs	No. hosts with eggs		Hosts with			
						1 egg	2 eggs	3 eggs	
25°C. 80 per cent. R.H.	A	38	43	30		17	13	—	
	B	36	43	33		24	8	1	
	C	36	40	29		18	11	—	
	D	40	41	30		19	11	—	
		Means	37·5	41·7	30·5	Totals	78	43	1
30°C. 80 per cent. R.H.	*E	27	71	45		20	24	1	
	*F	27	40	21		6	12	3	
	*G	27	70	41		15	23	3	
	*H	25	44	27		11	12	3	
		Means	26·5	56·2	33·5	Totals	52	71	10
	I	25	64	43		25	15	3	
	J	35	68	38		12	22	4	
	K	35	102	61		24	33	4	
	L	25	41	24		10	11	13	
		Means	30	68·7	41·5	Totals	71	81	24

* Discarded because daughters were present owing to eggs being overlooked.

Results (Table V) show that *Cephalonomia* females live for about 5 weeks at 25°C. and 80 per cent. relative humidity and for about 4 weeks at 30°C. and 80 per cent. relative humidity when plenty of *L. ferrugineus* larvae are available.

Van Emden (1931) found that *C. gallicola* females lived for 58 days at 21.9°C., 64 days at 19.1°C. and 75 days at 18.7°C. (mean temperatures) when fed on host larvae.

Longevity of adults with sugar solution available.

Van Emden (1931) found that *C. gallicola* adults would feed on sugar solution and would then live for much longer than if given only water or no food of any kind. Females lived for 50-79 days (58 days at a mean temperature of 21°C.) and 2 males for 20 and 41 days respectively.

Sugar solution is very difficult to supply to *Cephalonomia* because the insect is so small that it very easily sticks to the solution and perishes. A small experiment was, however, carried out with fairly satisfactory results in which both males and females were seen feeding on a supply of sucrose solution.

Three series of 2×1 in. tubes were set up at 30°C. and 80 per cent. relative humidity, (a) empty, (b) with a small piece of cotton wool at the bottom and (c) with a small piece of filter paper at the bottom. Sucrose solution was supplied daily. Small drops were placed on the cotton wool or filter paper or in the case of the empty tubes in tiny droplets on the walls and floors. Males or females (0–1 day old) were placed in these tubes and examined daily.

The results in series a were that 2 males had a range of longevity of from 9 to 13 days, with a mean of 11 days, 9 females had a range of 24 to 36 days with a mean of 29; in series b, 8 females had a range of 24 to 44 days with a mean of 35 days and in series c, 2 males had a range of 6 to 9 days with a mean of 7 days and 7 females 22 to 40 days with a mean of 30 days.

Most of the females appeared to die from "natural causes" and the results are seen to be very similar to those for the longevity of females fed on *Laemophloeus* larvae. The number of males used in this experiment was very low but three of them appeared to have died naturally after 6, 8 and 12 days respectively. The fourth stuck to the solution after nine days. In the previous experiments, in which males were starved, none lived for longer than six days. It seems conclusive, therefore, that the length of adult life of the males, as well as the females, is increased by feeding on sugar solution.

Pre-oviposition period and numbers of eggs laid in first 12 hours of oviposition.

Cocoons from cultures kept at 25°C. and 80 per cent. relative humidity were placed either singly or in small batches in 2×1 in. tubes and examined daily. When females emerged they were mated (in every case with a male from the same lot of cocoons) and placed with five *L. ferrugineus* larvae in small tubes varying from 2×½ in. to 1½×¾ in. which were stoppered with cotton wool. The *Laemophloeus* larvae were three weeks old and had been reared at 30°C. and 80 per cent. relative humidity. Fresh host larvae were added during the course of the experiment when all or most of those already present were paralysed or dead. The latter were removed at the time of examination. Sufficient larvae were added to ensure that there were at least two normal, actively moving host larvae in each tube at the beginning of each 24-hourly period after examination. Usually there was a total of 5–10 host larvae (normal+paralysed) in each tube. *Cephalonomia* will oviposit on larvae which have been paralysed some time previously.

A similar experiment was carried out at 30°C. and 80 per cent. relative humidity using exactly the same methods with the exception that no additional host larvae were needed during the course of the experiment.

The pre-oviposition period may be calculated in two ways from the data available. It may be taken as the period from emergence from the cocoon until the first egg is laid or from the time host larvae are available until oviposition, as the female probably requires a meal before she will oviposit. The periods calculated in both of these ways are given for each temperature.

Results are shown in Tables VI and VII.

At both 25 and 30°C., each parasitised *Laemophloeus* larva had 1, 2 or 3 eggs laid on it. The proportions of these groups laid during the first 12 hours of oviposition at each temperature were at 25°C., 31 larvae with 1 egg, 25 with 2 and 1 with 3; at 30°C., 17 larvae with 1 egg, 16 with 2 and 2 with 3. The period of 12 hours represents the mean of the range 0–24 hours between successive examinations during which eggs may have been laid.

TABLE VI.

Pre-oviposition period and numbers of eggs laid in first day of oviposition at 25°C. and 80 per cent. R.H.
 (a) Mean period in days from emergence from cocoon to oviposition.

Number of days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean pre-oviposition period
Mean range ...	From 0.5- To 1.5	1.5- 2.5	2.5- 3.5	3.5- 4.5	4.5- 5.5	5.5- 6.5	6.5- 7.5	7.5- 8.5	8.5- 9.5	9.5- 10.5	10.5- 11.5	11.5- 12.5	12.5- 13.5	13.5- 14.5	14.5- 15.5	5.2
Number of ♀♀ which oviposited	—	3	6	9	10	2	3	5	1	2	—	—	—	—	1	
Number of eggs laid	—	7	16	16	17	3	6	10	2	5	—	—	—	—	2	Mean number of eggs per ♀
Mean number of eggs laid during each period	—	2.3	2.7	1.8	1.7	1.5	2	2	2	2.5	—	—	—	—	—	2.0
(b) Mean period in days from addition of host larvae to oviposition.																
Days ...	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	
Range ...	0 (0-1)	1 (2)	2 (2-3)	3 (3-4)	4 (4-5)	5 (5-6)	6 (6-7)	7 (7-8)	8 (8-9)	9 (9-10)	10 (10-11)	11 (11-12)	12 (12-13)	13 (13-14)	14 (14-15)	Mean pre-oviposition period
Number of ♀♀ which oviposited	—	3	6	9	10	2	3	5	1	2	—	—	—	—	1	4.9

TABLE VII.

Pre-oviposition period and number of eggs laid in first day of oviposition at 30°C. and 80 per cent. R.H.

(a) Mean period from emergence to oviposition.

Number of days	...	1	2	3	Mean pre-oviposition period.
Mean range	...	(0.5-1.5)	(1.5-2.5)	(2.5-3.5)	1.7
Number of ♀♀ which oviposited		7	12	1	Mean number of eggs per ♀.
Number of eggs laid	...	17	35	3	2.6

(b) Mean period in days from addition of host larvae.

Number of days	...	0.5	1.5	2.5	Mean pre-oviposition period.
Range	...	(0-1)	(1-2)	(2-3)	1.2
Number of ♀♀ which oviposited		7	12	1	

The number of eggs laid by each female in the first 12 hours of egg laying was that at 25°C. and 30°C., respectively, 17 and 5 laid 1 egg, 14 and 5 laid 2, 7 and 6 laid 3, 0 and 2 laid 4, 3 and 2 laid 5, none laid 6 eggs, but one female at 30°C. laid 7 eggs.

These results may be summarised as follows :—

(i) At 30°C. and 80 per cent. R.H. the mean pre-oviposition period was 1.7 days (emergence from cocoon to oviposition) or 1.2 days (time of addition of host larvae to oviposition).

(ii) At 25°C. and 80 per cent. R.H. the comparable periods were 5.2 days and 4.9 days.

(iii) The range of the pre-oviposition period at 25°C. and 80 per cent. R.H. was 1.5-15.5 days (or 1-15 days).

(iv) The range of the pre-oviposition period at 30°C. and 80 per cent. R.H. was 0.5-3.5 days (or 1-3 days).

(v) At 25°C. and 80 per cent. R.H. most females laid 1 or 2 eggs during the first 12 hours of egg laying. The numbers laying 1 or 2 eggs were about equal.

(vi) At 30°C. and 80 per cent. R.H. most females laid 1, 2 or 3 eggs during the first 12 hours of egg laying. The numbers laying 1, 2 or 3 eggs were about equal.

(vii) Under both sets of conditions, the vast majority of females laid 1 or 2 eggs on each host larva parasitised. A few laid 3 eggs but none more than 3 per larva (4 eggs have been seen at other times, but very rarely). At 30°C., the numbers of single eggs and pairs of eggs were equal but at 25°C. more singles than pairs were laid. (Fuller data are given on this point below where a relation between sex ratio and number of eggs per larva is pointed out.)

Fecundity and egg-laying rate.

The numbers of eggs laid by each female in the experiment described on p. 87 (longevity of adults with host larvae available) are shown in Table V. Parasitised larvae were removed at each examination.

Cephalonomia females often abandon host larvae without laying eggs on them but on the other hand they lay eggs on larvae which have been paralysed or even parasitised previously by themselves or by other females. Their behaviour pattern from attack to oviposition may, therefore, be halted before oviposition has taken place, but it is not known at what stage it begins when a paralysed larva is selected for oviposition. It is possible that the already paralysed larva is stung once more and the whole pattern repeated but this seems unlikely. No cases of *Cephalonomia* stinging paralysed larvae have been recorded. That females will oviposit on larvae which have been paralysed or even bear eggs laid by other females was shown by enclosing them with paralysed or parasitised larvae on which they soon laid eggs. It is not claimed that oviposition on host larvae already bearing eggs is a normal procedure but confirmation that paralysed larvae are oviposited on when normal larvae are also available is obtained from a study of the egg-laying record of several of the females used in the longevity and fecundity experiments. A typical example is given in Table VIII.

TABLE VIII.

Numbers of host larvae paralysed and parasitised by one female at 30°C. and 80 per cent. R.H.

Period of days between examinations	Paralysed	Parasitised	Numbers attacked	
			Since previous observation	Since first day of experiment
1	—	—	—	—
1	4	—	4	4
2	16	3	15	19
3	21	9	14	33
4	21	9	9	42
3	28	3	10	52
4	21	8	1	53
4	16	11	6	59
3	27	—	11	70

N.B.—Parasitised larvae were removed at each examination : paralysed larvae were left in.

It will be seen that during two of the periods, viz., 14th–18th day and 18th–22nd day more host larvae were parasitised than were attacked. This shows that larvae which had been paralysed in previous periods must have been selected for oviposition.

In the fecundity experiments the number of eggs laid on each host larva was noted. The egg groups per larva were 1, 2 or 3. The numbers of larvae with each group are shown in Table V. It will be seen from the totals that at 25°C. more single eggs than pairs were laid, but at 30°C. more pairs than singles were laid. At 30°C. three or four trios were laid by all except one female (E) which laid only one. At 25°C. only one female (B) laid a trio.

The conclusions may be summarised as follows (see Table V).

(i) At 25°C. and 80 per cent. R.H. 41.7 eggs were laid in a life span of 37.5 days on 30.5 larvae (mean values). Ranges were 40–43 eggs in 36–40 days on 29–33 larvae. The mean egg laying rate was 1.11 eggs per day.

(ii) At 30°C. and 80 per cent. R.H. 68.7 eggs were laid in a life span of 30 days on 41.5 larvae (mean values for females I–L which completed life span). Ranges were 41–102 eggs in 25–35 days on 24–61 larvae. The mean egg laying rate was 2.29 eggs per day.

(iii) At 25°C. more single eggs were laid than pairs; the opposite was the case at 30°C. This difference in ratio of single to pairs will result in the production of a higher proportion of females at the lower temperature (see later section on sex determination).

(iv) *Cephalonomia* females will lay eggs on larvae which have been already paralysed or parasitised by themselves or by other females.

Parthenogenesis.

Virgin females, obtained from isolated cocoons, were kept at 25°C, 30°C. and 80 per cent. R.H. and *L. ferrugineus* larvae supplied.

At 25°C. and 80 per cent. R.H. 19 eggs were laid (singles and pairs). From these eggs 9 adults were obtained, all males. Several adults were lost before they were sexed. Mortality was 31.6 per cent. In the life-history experiment at 25°C. and 80 per cent. R.H. (Table III) mortality was 36.5 per cent.

At 30°C. and 80 per cent. R.H. 36 eggs were laid (singles, pairs and a trio). From these eggs 29 adults were obtained, all of them males. Mortality was 19.2 per cent. In the life-history experiment at 30°C. and 80 per cent. R.H. (Table III) mortality was 20 per cent.

Conclusions.—

- (i) Parthenogenesis can occur in *C. waterstoni*.
- (ii) Unfertilised eggs produce males (arrhenotoky).

Sex ratio.

Observations on the offspring of mated females showed that there was a tendency for single eggs to produce mainly females and for pairs of eggs to produce one male and one female. This was checked by isolating host larvae bearing one or two eggs. Larvae with three eggs were also used. The offspring were sexed. The sex of the adults obtained in the life-history experiments (Table III) was also determined and the results are incorporated in Table IX.

The results show that the majority of single eggs do in fact produce females and that the majority of pairs produce a male and a female.

From the majority of the trios, two females and one male are produced. It seems quite probable that in these cases a pair had been laid (♂♀) and then a single egg (♀) added afterwards, perhaps even by a different female.

From these results it appears that the chances of a single egg producing a female are about 8 : 1 and of a pair producing a male and a female about 5 : 1.

The same phenomenon has been recorded by Powell (1938) for *C. tarsalis*. Powell described the positioning of the eggs of each pair, the first on the prothorax which develops into a female and the second on the mesothorax which develops into a male. In *C. waterstoni*, the eggs are laid on any region of the ventral surface or the sides of the larva. The paralysed larva usually lies on its back and so the placing of the eggs ventrally is probably fortuitous. A few have been found with eggs on the dorsal surface.

Since it has been shown in the previous section that unfertilised eggs always develop into males, it seems quite likely that fertilisation always determines the sex. Probably eggs laid singly are always fertilised and thus develop into females, whereas when a pair of eggs is laid only the first is fertilised and the result is one female and one male. This is the explanation given by Powell for *C. tarsalis*.

Viability on three species of Laemophloeus.

Cephalonomia will accept as host the four species of *Laemophloeus* which are commonly found in stored foods. No other species have yet been tried. One of

TABLE IX.
Sex of offspring of mated females.

Experiment	Conditions	Sex of offspring										Sex ratio $\frac{\text{♀♀}}{\text{♂♂}}$
		Singles		Pairs		Trios						
		♀	♂	♂♀	♀♀	♀♀	♂♂	♀♀	♂♂	♂♂		
Sex ratio	30°C./80 per cent. R.H. ...	26	1	18	5	—	—	9	2	1	2.0	
Life-history 	30°C./80 per cent. R.H. ...	10	2	8	1	—	—	3	—	—	2.0	
	Totals 	36	3	26	6	—	—	12	2	1	2.0	
Life-history 	25°C./80 per cent. R.H. ...	14	1	7	1	—	—	—	—	—	2.875	
Life-history 	25°C./60 per cent. R.H. ...	15	7	8	1	2	—	—	—	—	1.3	
Life-history 	30°C./60 per cent. R.H. ...	15	1	15	2	—	—	2	—	—	2.1	
	Sum totals 	80	12	56	10	2	2	14	2	1	1.968	

these species cannot be named at present (Lucas & Oxley, 1946). The three named species are *L. ferrugineus*, which has been used as host in all of the experiments already described in the present paper, *L. minutus* and *L. turcicus*. The last is mainly confined to flour and the other two to grain.

Cephalonomia shows a differential preference for these four species of *Laemophloeus*. *L. ferrugineus* and *L. sp. indet.* are attacked and oviposited on more readily than *L. minutus* which in turn is more acceptable than *L. turcicus*. When given a choice of all four species simultaneously, *Cephalonomia* attacks approximately equal numbers of *L. ferrugineus* and *L. sp. indet.*, few *L. minutus* and very few *L. turcicus*. Details of experiments on their graded preference will be published later. The earlier experiments carried out took as the criterion for selection the numbers of adult *Cephalonomia* produced by equal numbers of the different species of *Laemophloeus*, which had been exposed to the parasite in a common chamber. This is open to the objection that the differential production of parasite offspring might be due to a differential viability on the various host species. In order to test this hypothesis an experiment was set up, using the three named species as hosts. *Cephalonomia* females were enclosed with 10 larvae of one of the three host species and allowed to lay eggs. All larvae bearing eggs were removed and the parasites allowed to develop. The results obtained are shown in Table X.

TABLE X.
Viability of *C. waterstoni* on three species of *Laemophloeus*.

Species of <i>Laemophloeus</i>	Number of eggs	Number of offspring	Percentage survival
<i>L. ferrugineus</i>	55	47	85.45
<i>L. minutus</i>	33	25	75.76
<i>L. turcicus</i>	58	45	77.58

Results show that about 75 per cent. of the eggs laid on *L. minutus* and *L. turcicus* and about 85 per cent. on *L. ferrugineus* survive and produce adults. This difference of 10 per cent. in the results is not to be taken as indicating that *L. ferrugineus* is a more suitable host than the other two species because, at different times during the experiment, percentage values were worked out and considerable variation was found. In a batch of 25 parasitised *L. turcicus* larvae, for instance, only one parasite died giving a percentage survival value of 96 per cent. The handling which the experimental animals have to undergo is probably a very important factor in affecting their mortality rate. The results of this experiment show clearly, however, that a high percentage of *Cephalonomia* eggs develop and produce adults on all three species of *Laemophloeus* and that probably *Cephalonomia* is equally viable on all three. The small numbers of *Cephalonomia* produced in cultures of *L. turcicus* is due, therefore, to some factors other than the ability of the parasite to develop on it.

Summary.

The paper describes field and laboratory investigations on the bionomics of *Cephalonomia waterstoni*, a Bethyloid parasite of *Laemophloeus* spp. A table is given in which are listed all the Bethyloids attacking insect pests of stored products to which reference could be found in the literature.

An infestation of *Laemophloeus*, associated with two "hot spots" in Manitoba wheat, which supported a large population of *Cephalonomia* is described.

A simple technique for the laboratory culture of *Cephalonomia* is described.

The life-cycle of *C. waterstoni* with *Laemophloeus ferrugineus* as host has been worked out.

The lengths of egg, larval and cocoon (prepupal and pupal) stages at combinations of 25°C., 30°C. and 60 per cent., 80 per cent. R.H. are given. The egg and larval stages are short, lasting for about six days at 25°C. and four days at 30°C.

Within the limits used, the relative humidity appears to have no effect on the duration of development at any stage. On the other hand, temperature exerts a considerable influence; the life-cycle at 30°C. is completed in two weeks but at 25°C. it takes three weeks.

Again within the limits used, the mortality appears to increase with decrease in saturation deficit. Mortality ranged from 9 per cent. at S.D. 12.7 mm. to 36.5 per cent. at S.D. 5.0 mm.

Without food or water at all combinations of 25–30°C. and 60–80 per cent. R.H. adults live for about four days, with a range of 0.5–9.5 days. There is no difference between the sexes. Unexplained contradictory results were obtained in two experiments.

With normal or paralysed host larvae available at 30°C. and 80 per cent. R.H., males live no longer than when no food or water is available but females live for about five weeks at 25°C. and 80 per cent. R.H. and for about four weeks at 30°C. and 80 per cent. R.H.

Males fed with sucrose solution at 30°C. and 80 per cent. R.H. live for several days longer than when starved; females live for the same length of time as when fed with host larvae.

The pre-oviposition period at 25°C. and 80 per cent. R.H. is about five days; at 30°C. and 80 per cent. R.H. about one and a half days.

Fecundity. At 25°C. and 80 per cent. R.H., *Cephalonomia* lays about 40 eggs on 30 host larvae; at 30°C. and 80 per cent. R.H., about 65 eggs on 40 larvae.

Cephalonomia females readily oviposit on larvae that have been paralysed some time previously, and can be induced to oviposit on larvae already bearing eggs.

Virgin females produce only male offspring (arrhenotoky).

Eggs are laid in groups of one, two or three (rarely four) per larva. Single eggs produce mainly females; pairs produce mainly one male and one female; trios produce mainly one male and two females. At 25°C. and 80 per cent. R.H. more single eggs are laid than pairs; at 30°C. and 80 per cent. R.H. more pairs are laid than singles. This results in the production of a higher proportion of females at 25°C. than at 30°C. The incidence of trios at both temperatures is low.

C. waterstoni is equally viable on *L. minutus*, *L. ferrugineus* and *L. turcicus* but shows a marked preference for *L. ferrugineus*.

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References.

- *ASHMEAD, W. H. (1893). Bull. U.S. nat. Mus., no. 45, 463 pp.
- BRÈTHES, J. (1913). An. Mus. nac. B. Aires, **24**, p. 87.
- *BRIDWELL, J. C. (1919). Proc. Hawaii. ent. Soc., **4**, pp. 21-38.
- *BRIDWELL, J. C. (1920). *Ibid.*, **4**, pp. 291-314.
- *BRIDWELL, J. C. (1929). Psyche, **36**, pp. 119-120.
- CLAUSEN, C. P. (1940). Entomophagous Insects. 688 pp. New York and London, McGraw-Hill.
- DURRANT, J. H. (1921). Rep. Grain Pests Comm. roy. Soc., **9**, pp. 32-52.
- EMDEN, F. VAN (1931). Z. Morph. Oekol. Tiere, **23**, pp. 425-574.
- *FOOTS, R. M. (1920). Proc. ent. Soc. Wash., **22**, pp. 61-73.
- GAHAN, A. B. (1931). J. Wash. Acad. Sci., **21**, pp. 213-221.
- GUSSAKOVSKIĬ, V. V. (1935). Rev. Ent. URSS, **25**, pp. 229-231. (Rev. appl. Ent., (A) **24**, p. 507.)
- KEARNS, C. W. (1934). J. econ. Ent., **27**, pp. 801-806.
- KEELER, C. E. (1929a). Psyche, **36**, pp. 41-44.
- KEELER, C. E. (1929b). *Ibid.*, **36**, pp. 121-127.
- LUCAS, C. E. & GLOVER, R. S. (1946). Ann. appl. Biol., **33**, pp. 293-302.
- LUCAS, C. E. & OXLEY, T. A. (1946). *Ibid.*, **33**, pp. 289-293.
- MATHER, K. (1946). Statistical analysis in biology. London, Methuen.
- MYERS, J. G. (1929). Bull. ent. Res., **20**, pp. 425-430.
- OXLEY, T. A. & HENDERSON, F. Y. (1944). J. Soc. chem. Ind., **43**, p. 48.
- OXLEY, T. A. & HOWE, R. W. (1944). Ann. appl. Biol., **31**, pp. 76-80.
- POWELL, D. (1938). Ann. ent. Soc. Amer., **31**, pp. 44-49.
- REID, J. A. (1942). Proc. R. ent. Soc. Lond., (A) **17**, pp. 27-33.
- RICHARDS, O. W. (1939). Trans. R. ent. Soc. Lond., **89**, pp. 185-344.
- RICHARDS, O. W. & HERFORD, G. V. B. (1930). Ann. appl. Biol., **17**, pp. 367-395.
- SARRA, R. (1930). Boll. Lab. Zool. Portici, **24**, pp. 223-227. (Rev. appl. Ent., (A) **19**, p. 249.)
- SCHREAD, J. C. & GARMAN, P. (1933). Bull. Conn. agric. Exp. Sta., no. 353, pp. 691-756.
- SHEPPARD, E. H. (1936). Tech. Bull. Colo. Sta., no. 17, 20 pp.
- VOUKASSOVITCH, P. (1924). Bull. Soc. Hist. nat. Toulouse, **52**, pp. 225-246.
- WATERSTON, J. (1921). Rep. Grain Pests Comm. roy. Soc., **9**, pp. 8-32.

*Not seen ; references from Richards (1939).

INSECT TRANSMISSION OF CACAO VIRUS DISEASE IN TRINIDAD.

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(PLATE II.)

Virus diseases of cacao have, until recently (Ciferri, 1948), been recorded only from the West Coast of Africa (Gold Coast, Nigeria, Ivory Coast and Togoland), where they are widespread and have been found "on all the main soils on which the crop is grown in West Africa" (Voelcker, 1948), and from Trinidad in the British West Indies. Ciferri (*loc. cit.*) states that in 1928 and 1929 he transmitted, by bud-grafting, a disease of cacao that he found in the Cibao valley in the Dominican Republic and he therefore now concludes that it is a virus disease, which he proposes to call "narrow wrinkled leaf" ("hoja estrecha abollada"). He considers it identical with one that he found more recently in the Cauca valley in Colombia. The symptoms of this disease which, when he first described it (1930) was thought to be "a disease of a degenerative type" and not a virus, seem to be entirely distinct from those of the cacao viruses occurring in West Africa and Trinidad. In West Africa more than 20 distinct "strains" of cacao virus have been recognised, some of which are certainly distinct viruses. In Trinidad only two "strains" have as yet been distinguished and it has not been satisfactorily determined whether these are different viruses or strains of one virus, the difference between a "strain" and a distinct virus disease being that infection by one strain can immunise a plant from infection by another strain of the same virus, whereas infection by one virus disease will not render a plant immune to infection by a distinct virus.

There are certain features that are common to these cacao viruses both in West Africa and Trinidad. The symptoms are in some respects very similar: these have been described for the West African viruses by Posnette (1944a) and by Posnette and others (West African Cacao Research Institute, Annual and Quarterly Reports, *passim*) and for the Trinidad viruses by Posnette (1944b), Baker and Dale (1947a, b), and also on p. 100 of the present paper. But the most important resemblance is that the insect vectors are exclusively, so far as is known, mealybugs of the family PSEUDOCOCCIDAE. Moreover, it is believed that this group of virus diseases is the only one definitely known to be carried by mealybugs. Four other records of virus diseases possibly carried by Coccids have been traced: bean mosaic by *Pseudococcus maritimus* Ehr. (Elmer, 1925) and by an undetermined mealybug (Fajardo, 1930); tobacco and tomato mosaic by *Pseudococcus citri* (Risso) (Olitsky, 1925); and a mosaic of the cactus, *Epiphyllum truncatum*, by *Orthezia insignis* Browne (Blattný & Vukolov, 1932). None of these appears to have been confirmed.

The fact that there are certain features in common may possibly indicate that these cacao virus diseases have been accidentally introduced from West Africa to Trinidad, or, though less probably, from Trinidad to West Africa. The second alternative is the less likely, since in West Africa cacao virus diseases have been known since 1930 (though not definitely recognised as virus diseases until 1940), and were probably present much earlier (Posnette, 1941) whereas in Trinidad the disease was first recognised in November 1943 and, judging from its present rate of spreading, is unlikely to have become established more than at most ten years before then.

The Trinidad cacao virus has less serious effects on the tree than some of the strains of "swollen-shoot" in West Africa, notably the virulent strain "A" (New

Juaben), which usually causes the death of the tree in from 12 to 24 months. No cacao tree in Trinidad is known with certainty to have died as a result of the virus, for although several infected trees in the Santa Cruz valley have recently died, owing to the generally bad condition of the cacao in some parts of this valley, it cannot be assumed that their death has been solely due to virus infection. Nevertheless, Baker and Dale (1947b) have adduced evidence that the virus is responsible for general unhealthiness—"small size, cessation of growth, dieback, 'stag-headedness', the development of small leaves and out-of-season flushing"—in the infected clonal cacao in the Diego Martin valley. Recently Cope (verbal communication) has analysed the figures for the yield (which has been recorded for the individual trees) of this field and has concluded that, on a mean of 15 pods per tree, there is a reduction of approximately two pods for every year that a tree has been infected.

A great deal of valuable work has been done in West Africa on the "swollen shoot" virus of cacao, which is the major problem that is being tackled by the West African Cacao Research Institute at Tafo in the Gold Coast. Very much less has been accomplished in Trinidad, as is only natural considering that here the problem has been tackled as a part-time project by the Professor and Lecturer of Mycology and latterly by the Professor of Entomology of the Imperial College of Tropical Agriculture, instead of by a research organisation devoted entirely to cacao problems. Nevertheless it seems desirable to put on record the facts that have so far been ascertained about insect transmission of the Trinidad cacao virus.

Description of the Symptoms.

Two strains, or possibly distinct viruses, occur in Trinidad. Posnette (1941) originally named these "Red-mottle" and "Vein-clearing". Baker and Dale (1947a) rejected these terms as names for the strains, and called them strain A and strain B respectively, since they found that strain A (Posnette's red-mottle) produces only "mosaic" on some varieties of cacao, while strain B (Posnette's vein-clearing) produces "red-mottle" as well as "mosaic" on certain varieties. However, they retained the terms "red-mottle", "mosaic" and "vein-clearing" as descriptive of the symptoms. It is suggested that these terms should be abandoned, both in order to conform with the terminology now in use at W.A.C.R.I. and also because they are not accurate descriptions of the symptoms. According to the Shorter Oxford English Dictionary "mottle" conveys the idea of spots or blotches of colour, whereas the "red-mottle" symptom is a continuous red feather-like banding at the sides of the veins. This symptom will therefore be called "red vein-banding". It is usually most conspicuous at the sides of the second-order veins* and on short lengths of the bases of the third-order veins, thus giving rise to a fern-like pattern. It also may occur at the sides of the midrib. The width of the red vein-banding is extremely variable: it may be so narrow as to require close scrutiny for its detection (Pl. II, fig. 1), or it may be as much as 5 mm. on each side of a vein. Occasionally it extends over all the lamina of a leaf or over a large part of it (Pl. II, fig. 2): it is then perhaps better described as "red-washing". This condition is rather more permanent than ordinary red vein-banding, which usually disappears completely before, often long before, the flush leaves turn dark green, and it may still be recognisable when the flush has almost completely hardened. However, after red vein-banding has faded, traces of its previous presence sometimes persist for a

* In conformity with the usage adopted at the West African Cacao Research Institute the veins of a cacao leaf are referred to as follows: The midrib is the primary or first-order vein; from it arise on either side some 8-12 second-order veins. Any two adjacent second-order veins are connected by an irregularly branched system of fairly conspicuous third-order veins, the smaller of which grade imperceptibly into the fine fourth-order veins, which are the smallest that can readily be distinguished by the naked eye. The lamina is everywhere minutely reticulated by numerous fifth-order veins, easily visible with a hand lens.

time in the hardened leaf in the form of a darker green vein-banding, due to the lamina between the fifth-order veins (which have now lost their red colour) being darker green than usual.

The symptoms of red vein-banding and red-washing are due to the conspicuous reddening of the normally pale pink fifth-order reticulum. Fourth- and third-order veins within an area affected by red-washing are also tinged red, but the colour of the lamina between the fifth-order veins is unchanged except that, as mentioned above, it tends to turn dark green about the time that the reddening of the fifth-order veins begins to fade. In cacaos that flush dark red, in which the fifth-order veins are themselves normally dark red instead of pink, the symptoms of red vein-banding and red-washing may perhaps be unrecognisable.

The "mosaic" symptoms of strain A are variable but usually take the form of more or less discontinuous yellow flecks at the sides of the veins, especially on the second- and third-order veins, but also on the midrib and fourth-order veins. This condition will be called "vein-flecking". The size and number of the chlorotic flecks may range from two or three isolated spots—in which case they occur most commonly near the tip of the leaf (Pl. II, fig. 3)—to continuous flecking along all or most of the veins, especially those of the second and third order (Pl. II, figs. 4, 5). On some cacaos the flecks tend to coalesce, particularly along the second-order veins (Pl. II, fig. 6), when the effect produced may be almost indistinguishable from the yellow vein-banding of strain B. Certain diagnosis is then only possible, as stated by Baker and Dale (1947a) by infecting either of the clones ICS 6 or ICS 60 with the suspect. If it was strain A it will produce on these clones red vein-banding only, whereas if it was strain B red vein-banding accompanied by vein-flecking will appear.

The symptom of vein-flecking is due to the lamina between the veins of the fifth-order reticulum being of a paler green, sometimes almost white, while the fifth-order veins themselves are yellow in a young cacao leaf in which they are normally pink, and somewhat enlarged and therefore more conspicuously yellow in a hardened leaf in which they are normally yellow.

It should be noted that the leaf symptoms produced by strain A are not necessarily identical on all the affected leaves of one tree, and that seedling* cacao trees in plantations, as well as young plants experimentally infected, show every possible gradation of symptoms from short lengths of narrow red vein-banding, with or without a few inconspicuous chlorotic flecks, to severe red vein-banding or red-washing extending over the whole leaf, and vein-flecking or chlorotic vein-banding on every vein of an affected leaf. Moreover, the symptoms on any one infected tree are not always the same on every flush. Baker (verbal communication) is of the opinion that they tend to be more severe towards the end of the dry season (January to May). Although I have kept no precise records on this point I agree with him at any rate as far as seedling cacao is concerned.

As stated by Baker and Dale (1947a) "strain A also causes red blotches on the pods of certain yellow-podded cacao varieties" (*i.e.* those in which the immature pods are green, not red). This condition, which only appears on the outer side of the pods exposed to a greater light intensity, could aptly be described as "red-mottle" of pods.

The term "vein-clearing" for the symptom commonly produced by strain B is also somewhat inapposite, since, as is evident from Baker and Dale's photographs the veins are seldom "cleared". Occasionally the veins themselves appear more yellow than usual, but as a rule the condition produced by strain B is a yellow banding at the sides of the second- and third-order veins. This symptom will therefore be called

* The term "seedling cacao" means cacao trees propagated from seed and has nothing to do with the age of the plants.

"yellow vein-banding". Strain B also gives rise, on some cacaos, to a certain amount of red vein-banding.

Neither "green-mottle" of pods, *i.e.* blotches of darker green on young green pods, nor "round-pod", a malformation of the pod in which it becomes much more shortly oval than usual, nor "swollen-shoot", *i.e.* conspicuous swellings on chupons or fans, all of which are symptoms produced by some of the West African cacao viruses, have been observed in Trinidad. There appears to be little if any differentiation of symptoms into an "acute" stage—the first to appear, and a "chronic" stage—the less conspicuous symptoms manifested after a tree has been infected for some considerable time, as has been recorded for the West African cacao viruses (Voelcker, 1946). Certainly most if not all of the trees in the Santa Cruz valley that were marked as infected by Baker and Dale in 1944 still show very severe symptoms.

The Species of Mealybugs found on Cacao in Trinidad.

I am indebted to Dr. W. J. Hall, Director of the Commonwealth Institute of Entomology, for identifying or confirming my identifications of the following species of mealybugs.

(1) *Pseudococcus citri* (Risso). This is the commonest species of mealybug on cacao in Trinidad and is no doubt responsible for most of the natural spread of the virus. It has been recorded by Fennah (1947) from many host plants in the Lesser Antilles, including cacao, cassava, citrus, coffee, guava, soybean, celery, *Solanum* sp. and *Ipier* sp., also from the roots of cowpea. Wolcott (1933) records it on coconut, and I have found it in Trinidad on *Gliricidia sepium*. I have never seen it on citrus, nor can I find any record of its having occurred on citrus in Trinidad. Specimens of all ages transferred from cacao to the leaves and stems of young citrus plants failed to survive: it can, however, feed and reproduce on the unripe fruits of sweet orange. The duration of the egg stage seems to be very variable, from four to nine days: the complete life-cycle (*i.e.*, from oviposition to oviposition by females of the next generation) in the laboratory, takes 27 days upwards. Males are produced abundantly and, as was first demonstrated by James (1937), fertilisation of the female is essential for reproduction. On cacao the largest colonies are found on the pods, less often on chupons. A cacao tree frequently harbours numerous small colonies at the base of flowers and young cherelles, which are often destroyed as a result. Single individuals, but seldom colonies, are often found on leaves. It appears to be more abundant in the drier cacao areas and during the dry season (January to May). Though large colonies of this species are invariably attended by ants in the field (single individuals and very small colonies may not be) it thrives when bred on potato tubers in the laboratory unattended by ants. In West Africa, *P. citri*, though not common on cacao, has been proved to be a vector of all the viruses that occur there.

(2). *Pseudococcus brevipes* (Ckll.). Dr. W. J. Hall (*in litt.* 1948) stated that one batch of specimens did not appear to be quite typical of this species, the dermal spines in the anterior region being stouter than usual. Further specimens were sent to him, and on these he reported that the dermal spines anteriorly were less stout and the specimens seemed to be more typical of *P. brevipes*. This mealybug has been recorded by Fennah (1947) in the Lesser Antilles on maize, pineapple, sugar cane, tamarind, pomegranate, and the roots of soybean, bananas and *Cyperus rotundus*. It is abundant on pineapple in Trinidad, but not very common on cacao, though occasionally large colonies occur on pods or on the bark near the petiole of a pod: I have not noticed it on chupons or leaves. It always appears to be attended by ants that build a more or less complete "tent" over the colony.

This is a viviparous mealybug, which can complete its life-cycle in 24 days, though the time taken is usually somewhat longer. Males are common and fertilisation certainly takes place as a rule. I have, however, recorded one instance of apparent

parthenogenesis. A few colonies, bred on potato tubers in the laboratory, produced no males and the colonies died out. Similar cases of families consisting entirely of females have been recorded by James (1937) for *P. longispinus*.

Recently Posnette (*in litt.* 1949) has informed me that *P. brevipes* has now been proved a vector of virus 1A in the Gold Coast.

(3). *Pseudococcus* sp. near *brevipes*. This is a species that Hail (*in litt.* 1949) considers distinct from *P. brevipes* (Ckll.) for the following reasons. "It has fewer spines to the cerarii and the group of trilocular pores associated with each cerarius is smaller and less dense. The trilocular pores elsewhere on the dermis also are more scattered and less numerous. The hind limbs, in particular the tibiae, are less stout and translucent pores are entirely lacking on the hind tibiae and femora whereas in typical *brevipes* they are numerous." So far I have only found this species on cacao, though it will live readily on potato tubers. Superficially it is a less convex insect than *P. brevipes* and has longer wax tassels, but it is noteworthy that if *P. brevipes* is bred on potato without ant attendants, or with a species of ant that does not build a "tent" over it, it tends to resemble this species in general appearance. Though not common, it occasionally occurs in large colonies on pods, attended by ants that do not build a "tent". I have seen it on chupons but not on leaves or on the bark. Males are common and are apparently necessary for reproduction: unfertilised adult females have lived for over three months but have produced no offspring. The female is viviparous: in the laboratory, when fed on potato, females have become adult in 26 days though there has then usually been a period of three weeks or more before nymphs were produced. Observations indicate, however, that this is not so in the field, and that females can give birth to nymphs soon after becoming adult.

(4). *Ferrisia virgata* (Ckll.). Recorded by Fennah (1947) in the Lesser Antilles on asparagus, hibiscus, egg-plant, beans, *Plumeria* and *Codiaeum* sp., and by Wolcott (1933) on cotton and coconut. I have also seen it abundant on *Gliricidia sepium* in Trinidad. On some host plants it forms large colonies but on cacao it usually occurs only in small colonies or as isolated individuals, either on pods, or—more often than the other species—on leaves. This also is a viviparous mealybug: the complete life-cycle can, in the laboratory, be undergone in 36 days. It is apparently never attended by ants. It was with this species that the first definite transmission of the Trinidad cacao virus by an insect vector was obtained by Dale.

(5). *Pseudococcus longispinus* (Targ.). This species has so far failed to carry the Trinidad cacao virus, though it is noteworthy that in West Africa it is a vector of two strains, C (Kpeve) and M (Mampong), which are the only two strains that are not, in the Gold Coast, transmitted by *Ferrisia virgata* (Posnette, 15th Quarterly Report, West African Cacao Research Station, Tafo, 1948). It has been recorded by Wolcott (1933) as occurring on cotton in the West Indies although, apart from cacao, I have so far only definitely found it on *Gliricidia sepium*. It is oviparous, but the eggs hatch within a few hours of being laid, and both sexes become adult in about 22 days. Males are common and according to James (1937) fertilisation is essential for reproduction. Though widely distributed on cacao in Trinidad it is seldom common, usually occurring as single individuals or in small colonies on pods or leaves.

Seventy-four test beans, on which a total of 309 potentially infective mealybugs of this species had settled and apparently fed, have been grown but none of the resulting plants developed any symptoms.

(6). *Puto barberi* (Ckll.). This is a mealybug that is common on numerous garden plants and on *Gliricidia sepium* used as shade for cacao. I have only seen it established on cacao growing under *Gliricidia* and though it seems to be able to live and reproduce perfectly well on cacao, its occurrence thereon is probably purely accidental. So far, I have failed to transmit the virus with this species: 37 plants have been grown from beans on which a total of 113 infective mealybugs settled, but no transmission resulted.

(7). *Orthezia insignis* Browne. This is a cosmopolitan species, belonging to the family ORTHEZIIDAE, that occurs on a wide variety of host plants. As is the case with *Puto barberi* it does not normally feed on cacao, only becoming established on it when the cacao is grown under *Gliricidia sepium*. It is apparently not a vector of the virus, judging from 22 test plants, on which a total of 114 infective individuals had settled, but none of them became infected.

(8). In addition to the foregoing I have recently found another species, probably a *Pseudococcus*, occurring sparingly on cacao at St. Augustine. This has not yet been identified nor have any transmission experiments been made with it.

(9). A few transmissions have also been attempted with a species of *Phenacoccus* that was found in abundance on *Hibiscus mutabilis*. This has not been observed on cacao although when transferred experimentally it feeds readily. A total of 137 potentially infective mealybugs of this species were transferred to 23 test beans, but no transmission was obtained.

A small number of transmission tests has been made with Aphids (probably *Toxoptera aurantii* Boyer) but, as was found to be the case in West Africa (Posnette & Strickland, 1948), they all proved negative. Possible vectors that have not yet been tested are ALEURODIDAE: a preliminary attempt to establish colonies of the common cacao white-fly on young caged cacao plants was unsuccessful.

Methods employed in Transmission Experiments.

Most of the experiments so far have been made with *Pseudococcus citri* as vector, since this is certainly the mealybug responsible for most of the natural spread of the virus in Trinidad. This species is, therefore, to be understood except where another is specifically mentioned.

In nearly all experiments strain A has been used as the source of the virus, chiefly because it is much more readily obtainable. Baker and Dale (1947a) stated that strain B occurs in the Maracas valley and parts of the Santa Cruz valley. In the latter all, or at any rate most, of the infected trees have been eradicated. There is still a considerable number of trees showing symptoms closely resembling those of strain B, but all of these that have been tested, by infecting the clone ICS6 from them, have proved to be strain A, the only symptom appearing on the test plant being red vein-banding. Many of the trees in the Maracas valley infected with strain B have also been removed, though this strain can still be found there. The very few results obtained with strain B are mentioned separately (p. 113).

Posnette's (1947) technique of feeding potential vectors, after they have fed on the source of the virus, on one half of a cacao bean, has been adopted as a general practice. This method has numerous advantages, especially in the saving of time and greenhouse space, nevertheless several difficulties have been encountered that have reduced the value of the results so far obtained. It has been necessary to use cacao beans from such ripe pods as have been available, and it is possible that some of the inconsistent results may be due to the genetical impurity of Trinidad cacaos. Growth of the seedlings from dissected beans has often been extremely unsatisfactory: there has frequently been marked chlorosis and other obvious disorders that may quite possibly have masked any virus symptoms. It is possible that the comparative rarity of any symptoms of vein-flecking appearing in the experimental plants, most of which have shown red vein-banding only, may be due to this poor growth, for in naturally infected seedling cacaos in plantations some form of vein-flecking usually accompanies red vein-banding, or may be the only symptom.

It is probable that this trouble is at least in part due to the poor quality of the soil used and it is hoped to remedy this in future experiments by obtaining a quantity of a better cacao soil from central Trinidad.

Neither *P. citri* nor any of the other three Trinidad vectors "behave" in such an exemplary manner as *P. njalensis* Laing apparently does at Tafo in the Gold Coast. For it is said by Posnette and Strickland (1948) that if a number of *P. njalensis* are placed on a dissected cacao bean in a solid watch-glass, and if some should fall off the bean, they can easily get back again. I have found that *P. citri* certainly falls off the bean on which it has been placed very frequently, but that it very seldom succeeds in getting back. This, however, has a certain advantage: for if a number of mealybugs have been put on a bean and the number that has settled after say 15 minutes has been recorded, it can be assumed with reasonable certainty that only that number has fed on the bean, the rest will neither have fed on it nor will they subsequently climb back on to it to feed. The number of mealybugs that has actually fed can therefore be recorded with very fair accuracy with a minimum of observation.

Another way in which the Trinidad virus differs from those of West Africa, and which detracts somewhat from the value of the "bean-feeding technique" is in the "latent period" of the virus in the plant. Posnette and Strickland (1948) state that, at Tafo, when infective mealybugs are fed on a dissected cacao bean the symptoms of virus will appear, if the plant has become infected, usually on the first flush, about 17-25 days after planting the bean, or if not, on the second flush which is produced after some 40-50 days. If symptoms have not shown up on the second flush the plant can be said definitely not to have been infected and can therefore be discarded, thus avoiding the necessity of keeping large numbers of plants for long periods. This is not so with the Trinidad virus. It is exceptional for symptoms to appear on the first three or four leaves produced: though they quite frequently show up on the next few leaves many instances have been recorded when no visible symptoms have appeared until much later (Table I). Young seedlings of Trinidad cacao plants, grown in boxes in a greenhouse, can hardly be said to "flush" in the manner of older cacao plants: they usually produce one or sometimes two new leaves at intervals, often very irregular, of about a week or ten days.

Notwithstanding these drawbacks, Posnette's bean-feeding method clearly has many advantages over infecting young plants with mealybugs. The only experiments that have been made using the latter are as follows. With *P. citri*, eight transmissions out of 27 tests: ten nymphs were put on each plant, but the number that settled was not recorded. Six out of 13 of these were with first-instar nymphs. With third-instar nymphs of *P. brevipes*, three transmissions resulted from 17 tests: with third-instar nymphs of *F. virgata* four transmissions from 28 tests. In these transmissions the symptoms appeared 27 to 81 days afterwards. There does not, therefore, seem to be any obvious difference either in the percentage of transmissions or in the "latent period" in the plant, from that observed in experiments in which the mealybugs are transferred to dissected beans.

Source of Mealybugs used in Transmission Experiments.

In some transmission experiments, mealybugs have been collected from pods and leaves showing virus symptoms, and then used direct; in others, they have been collected from healthy cacao trees and then fed on a virus source plant. The most usual method, however, has been to breed up a supply of mealybugs, originally collected from cacao, on potato tubers and then feed them on the source plant. All the species of mealybugs with which transmission experiments have been attempted feed readily on potatoes, especially those having a few incipient sprouts, and it has not been found necessary to germinate the tubers in order to produce large sprouts. This has proved the most convenient method to ensure always having a supply of mealybugs of any desired age readily available. Although the three vector species of *Pseudococcus* (*citri*, *brevipes* and sp. near *brevipes*) are, when living naturally on

cacao in Trinidad, almost invariably attended by ants (though *Ferrisia virgata* is not) colonies of all these species can readily be established on potatoes without ant attendants. There is no more difficulty in getting them to feed when transferred from cacao to potatoes, or from potatoes to the cacao source plant, than there is when they are transferred from the source plant to the test plant or test bean. They are, moreover, much more easily dislodged from potato tubers than from cacao (especially from cacao pods) without damage to their mouth-parts. Only those mealybugs that have withdrawn their mouth-parts and started to walk have been used and if a sufficient number have not withdrawn their stylets voluntarily they can generally be induced to do so by a slight touch on the dorsum with a camel-hair brush. I have succeeded in removing mealybugs, while they were actually feeding on soft tissue such as a potato tuber, without damage to the extruded stylets, and they have been able to retract into the crumena and subsequently to feed again. But one cannot guarantee that the mouth-parts will not be damaged and further feeding thus rendered impossible.

Sources of Virus Used.

Except in experiments designed to ascertain the efficiency as a source of the virus of symptom-free parts of infected cacao trees (p. 112), the source employed has usually been symptom-showing flush leaves, either those of small growing plants that had been infected in previous experiments, or flush leaves picked from old diseased trees in a plantation and kept fresh by inserting the petioles into a tube of water. There appears to be no difference in the efficiency of these two sources. Pods showing the symptom of red-mottle were used in a few early experiments and 12 transmissions obtained out of 63 tests: for all later experiments they were discarded in favour of flush leaves, owing to the greater difficulty of getting the mealybugs to withdraw their mouth-parts uninjured from pods.

"Latent" Period of the Virus in the Test Plant.

Table I shows the "latent period" of the virus for all plants that have been infected with strain A by all four vectors, by means of the bean-feeding technique, in experiments completed up to May 1949.

TABLE I.

Days after infection by mealybugs	Number of instances of first symptoms of	
	Red vein-banding	Vein-flecking
20-29	22	1
30-39	68	6
40-49	97	12
50-59	26	4
60-69	17	1
70-79	12	4
80-89	7	2
90-99	3	1
100-109	4	3
110-119	1	4
120-129	—	2

Forty-nine out of the total of 297 infected plants enumerated in Table I eventually showed symptoms of both red vein-banding and vein-flecking. In only two of these the vein-flecking appeared first, in one case 11, and in the other 47 days before the red vein-banding. In 14 plants both types of symptom appeared on the same day

(these are included in the column "red vein-banding" in the table). In the remaining 33 the symptom of vein-flecking appeared later than that of red vein-banding by 1-10 days (13 times); 21-30 days (5 times); 31-40 days (5 times); 41-50 days (5 times); 51-60, 61-70, 71-80, 81-90, and 121-130 days, once each.

Type of Symptoms appearing on Experimental Test Plants.

Although in plantations, in seedling cacao trees that have become naturally infected with strain A, some form of yellow vein-flecking usually accompanies red vein-banding, at any rate on some of the symptom-showing leaves (according to Baker and Dade (1947a) it is only on a few clonal cacaos such as RCS6, 53 and 60 that yellow vein-banding does not appear), in the transmission experiments so far carried out the symptom of vein-flecking has appeared seldom compared with that of red vein-banding.

Table II shows the number of times the different symptoms of strain A have appeared on the test plants, for all cases of laboratory transmission by mealybugs (all four vectors) in which the symptoms on the source plant were recorded—in some earlier experiments they were not recorded, hence the slightly lower totals in this Table than those in Table I.

TABLE II.

Symptoms on source plant	Symptoms on test plant		
	Red vein-banding only	Red vein-banding plus vein-flecking	Vein-flecking only
Flush leaves with red vein-banding only	105	20	18
Flush leaves with red vein-banding plus vein-flecking ...	82	13	8*(6)
Flush leaves with vein-flecking only	12	3	7*(2)
Red-mottled pod	8	2	2

* The apparent preponderance of vein-flecking in the few test plants infected from a source with vein-flecking only may possibly be explained by the inclusion of an experiment in which test beans from the Surinam cacao M.8 were used. Seven plants grown from these beans became infected and all of them showed vein-flecking only. Five of them were infected from a source that showed vein-flecking only and two from a source that showed vein-flecking and red vein-banding. It may be that seedlings grown from beans of M.8 can only show the symptom of vein-flecking and cannot produce red vein-banding, in which case the results of the experiment with M.8 should be omitted from the above, and the figures in brackets substituted.

The results shown in Table II may merely indicate that with strain A the symptoms shown by the source plant have no effect on those appearing in the test plant, which itself conditions the type of symptoms. It is, however, more than possible that the poor growth of many of the test plants has masked the symptom of vein-flecking, which would in many cases have appeared if the plants had grown in a normal manner without undue chlorosis.

Infectivity of the different Instars of the Mealybugs.

There is little difficulty in separating first-instar nymphs of *P. citri* from the second, and second-instar from the third, but it is not always easy to distinguish third-instar nymphs from young adults. Third-instar nymphs have only seven

antennal joints whereas the adult has eight, but this character is often impossible to see in the living insect, being obscured by fragments of wax and dirt. Some mistakes have therefore no doubt been made in separating these two instars, nevertheless it is reasonably certain that all four stages of *P. citri* are capable of transmitting the virus.

More experiments have been made with third-instar nymphs and young adults than with the two earlier stages, largely because the former are easier to handle. Table III gives the results of all experiments, except those series from which no positive transmissions were obtained, in which the instar of the mealybug and the number of mealybugs that settled on the test beans were recorded.

TABLE III.
Transmission of the virus by the different instars of *P. citri*.

Instar of the mealybug	Number of test beans	Number of test plants infected	Percentage of test plants infected	Average number of mealybugs settled per test bean
First	63	21	33.3	4.6
Second	69	15	21.7	2.5
Third	393	96	24.4	3.0
Young Adult ...	201	35	17.4	2.6

These figures do not appear to indicate any marked difference in the efficiency as vectors of the four instars, for the apparently higher percentage of transmissions obtained with first-instar nymphs may be due to the fact that on the average a larger number of them were used, as is shown in the last column. Young adults may perhaps be slightly less efficient transmitters than the immature stages.

Only a few transmissions have been attempted with older, parturient adults and these were all unsuccessful, possibly because the insects, although they settled, did not feed on the test beans.

There is no reason to suppose that transmission cannot similarly be obtained with all instars of the other three vectors. So far, however, it has only been proved with the following :—

P. brevipes : All four stages.

P. sp. near brevipes : Young adults and third-instar nymphs.

Ferrisia virgata : Young adults, third and second-instar nymphs. No transmissions have yet been attempted with the first and second instars of *P. sp. near brevipes* and the first instar of *F. virgata*.

The Effect of Starvation of the Vector before Feeding on the Source of the Virus.

With some aphis-carried "non-persistent" viruses, a much higher rate of transmission is obtained if the vectors are starved before feeding on the source plant (Watson & Roberts, 1939), and it has been suggested (Watson, 1946) that this effect of pre-source-feeding starvation should be regarded as the main basis of distinction between persistent and non-persistent viruses.

The Trinidad cacao virus is certainly "non-persistent" in the usual sense of this term ; i.e., the vectors lose their infectivity comparatively soon after ceasing to feed on the virus source (as is shown on pp. 111) but pre-source-feeding starvation does not appear to make the mealybugs more efficient vectors.

Provided that unstarved mealybugs settle and feed on the source, they seem able to transmit the disease as readily as those that have been starved. While no strictly comparable experiments have yet been made, in a number in which the mealybugs were transferred direct to the test beans either from infected cacao in the field or from plants previously infected in the laboratory, the proportion of transmissions has been as high as the average. However, in most of my experiments the mealybugs have been starved for a period varying from 6 to 72 hours before being fed on the source, because if this is done they usually settle better on the source. If they have not been starved a large proportion often walks off the source and therefore has to be rejected. Pre-source-feeding starvation does not, however, always have the desired effect of making the mealybugs settle readily on the source: sometimes, for no obvious reason, the proportion settling has been very low. Third-instar nymphs and young adults can endure starvation for more than three days, but apparently settle and feed less readily if starved too long: 12-24 hours is probably the optimum starvation time to encourage good settling.

The Effect of Length of Feeding Time on the Virus Source.

According to Watson (1946) the efficiency as transmitters of some aphid vectors of "non-persistent" viruses decreases with the time they have spent feeding on the source, the highest percentage of transmissions being obtained after only a very short (two to five minutes) period of source-feeding. This does not appear to be the case with the mealybug vectors of the Trinidad cacao virus. The shortest time in which a mealybug has become infective is 33 minutes, and 5 transmissions out of 16 tests have been obtained with periods varying between 33 and 60 minutes, using an average of nine mealybugs per test bean. Only 7 tests have been made, also with an average of nine mealybugs each, for periods between 15 and 30 minutes, but in none of these was transmission obtained.

Table IV, which includes all transmission experiments so far made except (1) some "blunderbuss" ones designed merely to ascertain if certain species were

TABLE IV.

Time on source plant	Vector Species				All four vectors
	<i>P. citri</i>	<i>P. brevipes</i>	<i>P. sp. nr. brevipes</i>	<i>F. virgata</i>	
$\frac{1}{2}$ -2 hours ...	24/129* =18.6 per cent.	2/4	—	3/16	29/149 =19.6 per cent.
2-6 hours ...	81/323 =25.1 per cent.	10/27	11/35	2/12	104/397 =26.2 per cent.
6-18 hours ...	6/26	—	—	2/5	8/31
Over 18 hours ...	147/584 =25.2 per cent.	26/75	16/92	—	189/751 =25.2 per cent.
Total ...	258/1,062 =24.3 per cent.	38/106 =35.8 per cent.	27/127 =21.3 per cent.	7/33	330/1,328 =24.8 per cent.

* The first figure is the number of transmissions obtained, the second figure the number of tests made.

vectors or not, in which the duration of feeding on the source plant was not recorded, and (2) those experiments (e.g., post-infection-feeding before transferring to the test bean) in which no transmissions were obtained, indicates that the duration of the infection-feed has little if any effect on the efficiency of the mealybugs as vectors. The lower percentage of transmissions obtained with *P. citri* when the source-feeding period was less than two hours may perhaps be due to some of the mealybugs, although they had settled, not actually having fed. There is an apparent discrepancy in the results obtained by feeding *P. sp. near brevipes* for 2-6 hours, which are considerably higher than those for over 18 hours, but the number of tests is too small to be of much significance. It would, however, appear from these figures that *P. brevipes* may be a rather more efficient vector than the other three species.

It may be noted that the total percentage of transmissions with *P. citri*, 24.3 per cent., is not materially different from that given in Table III which included only experiments in which the number of settled bugs was recorded.

Feeding on the Test Bean.

Duration of test-feeding.

No adequate experiments have yet been made with short feeding periods on the test bean. The shortest times in which transmission has so far been obtained have been one of 90 minutes and one of 100 minutes out of a total of 11 tests for times between one and two hours, using an average of four *P. citri* per bean. Of 247 tests made with feeding periods of between 3 and 7 hours, 60, or 24.3 per cent., resulted in transmission. This percentage is identical with that obtained from the majority of transmission experiments with *P. citri*, in which the insects were left on the test beans for 18-24 hours.

It can therefore be tentatively concluded that the duration of the test-feed is immaterial—provided of course that the insects settle and do actually feed.

Feeding position on the test bean.

It was thought possible that the part of the dissected bean on which the mealybug feeds might have some influence on transmission. Single mealybugs (*P. citri*) were therefore placed on each test bean and their feeding position, whether on the cotyledon or on the radicle, was recorded. For some reason, possibly the very poor growth of some of the test plants, transmission in this experiment was below average. Four plants out of 88, on which the mealybug is known to have fed only on the cotyledon, became infected; and 3 plants out of 38 on which it had fed only on the radicle. All that can be concluded, therefore, is that transmission can be obtained whether the insect feeds on the cotyledon or the radicle of the test bean.

Proof that the mealybugs have actually fed on the test bean.

There is no way of proving whether a mealybug, even though it has settled, is actually feeding except to remove it forcibly and observe whether or not the stylets are extruded, and if this is done the insect can seldom be used again. However, failure to feed on the test bean is certainly not the main cause of failure to transmit the virus, since in 73 of the tests mentioned in the preceding paragraph, the mealybugs were examined at the end of the test-feeding period. Forty-eight of them were undoubtedly feeding, yet only 7 of these transmitted the virus. The other 25 were not feeding, and though of course they may have fed before, no transmission was obtained from these.

Starvation after Infection-Feeding.

TABLE V.

Post-infection-feeding starvation			Tests	Transmissions	Average number of mealybugs per test bean
			<i>P. citri</i>		
2-4 hours	47	11	4
5-9 hours	37	9	4
17-22½ hours	30	5	4
23-48 hours	30	0	4
			<i>P. brevipes</i>		
5-9 hours	23	8	7
23-48 hours	15	0	3

Table V shows the results obtained. It appears that, at any rate up to 9 hours, starvation has no effect on the ability of the mealybugs to transmit the virus. It is moreover unlikely that transmission after 17-22½ hours starvation is significantly lower since the few data obtained include 3 transmissions out of 6 tests after 17 hours and none out of 12 after 18 hours. In this latter experiment it seems likely that the failure was due to some unexplained cause, not the starvation, since transmission was also obtained in one out of four tests after 22 hours, and one out of four after 22½ hours starvation. The total number of transmissions after varying periods of post-infection-feeding starvation up to 22½ hours, viz., 25 out of 114 with *P. citri* and 8 out of 23 with *P. brevipes*, is very similar to that obtained for all experiments including those in which the mealybugs were transferred direct from the virus source to the test beans (Table IV). No transmissions, however, have resulted when the post-infection-feeding starvation was 23 hours or longer. No experiments on these lines have been made with *P. sp. near brevipes* or with *F. virgata*.

Feeding after Infection-Feeding.

Only one experiment has so far been made, in which mealybugs (*P. citri*) were fed on a healthy cacao pod of 66 hours after infection-feeding and then transferred to test beans, an average of five settling on each. No transmissions resulted: in the control to this experiment 12 out of 23 transmissions were obtained with a smaller average (3.5) of mealybugs to each test bean.

It is not therefore known whether a mealybug is capable of infecting more than one plant, or whether, having once fed after infection-feeding, it loses its ability to transmit. Experiments to ascertain this will probably have to be made on young test plants instead of beans, owing to the difficulty of finding out whether a mealybug has actually fed on a bean and then removing it without damage to its mouth-parts.

The Proportion of Infective Mealybugs.

Transmission of the virus can be obtained with a single mealybug, but it is by no means every mealybug that does transmit it, even when the best conditions for transmission—so far as they are known—have been fulfilled. As explained on p. 110, this is certainly not due to some of the insects not feeding on the test beans, for in several experiments they have been removed at the end of the test-feeding period and a note made whether or not the stylets were extruded. Many failures to transmit have been recorded when the insect was certainly feeding on the test

bean, as well as some successes when they were not feeding at the end of the test period, showing that they had fed previously.

Table VI shows the number of tests that have been made and the number of transmissions obtained for all cases in which the number of mealybugs that settled on the test beans was recorded. No satisfactory explanation can be offered for the fact that the proportion of successful transmissions does not rise with the number of vectors used, in accordance with mathematical expectation. For if 13.7 per cent. transmissions can be obtained with a single mealybug, 25.5 per cent. would be expected with two, 36 per cent. with three, 43 per cent. with four, and 50 per cent. with five. The observed figure for two mealybugs is very close to that expected but with increasing numbers it is much less. A somewhat similar anomalous result was obtained by Posnette and N. F. Robertson (12th Quarterly Report, West African Cacao Research Station, Tafo, 1947) using *P. njalensis* and strain M of the West African cacao virus. In a series of 60 tests each, with one mealybug per plant 8 transmissions occurred; with two mealybugs, 22; with three, 26; with five, 32; and with ten, 38.

A possible source of error, which, however, I made every effort to avoid, might be that more care was used in transferring one or two mealybugs to each test bean than when larger numbers were used, some of which might have had their mouth-parts damaged. I think it is more likely that the unsatisfactory and uneven growth of the test plants in some experiments may have precluded the appearance of symptoms. Thus the rate of infection by single mealybugs was undoubtedly biased by one series, in which the plants were all growing well, when 15 out of 63 transmissions were obtained, a proportion that has not so far been approached in subsequent experiments.

Another possibility, that might help to explain this and other anomalies in rates of transmission, is that there might be "active" and "non-active" races of *P. citri*, as in the case of the Jassid vector, *Cicadulina mbila* Naudé of Streak disease of maize (Storey, 1932).

So far I have practically no evidence for the occurrence of inactive races, for most transmission experiments have been made with mixed populations of mealybugs, either collected from cacao in plantations or the mixed offspring of several females bred on potatoes. But it is noteworthy that one series of transmission experiments has been made with the offspring of three female *P. citri*, bred separately, which were the inbred descendants of a single female taken from a cacao tree. All of these failed to transmit: the first to four test beans with ten mealybugs each and to 15 with one; the second to one bean with ten mealybugs and to eight with one; the third to one bean with ten and to 11 with one. There was no obvious reason why there should have been no single case of transmission in this series comprising six test plants with ten mealybugs each and 34 with one, unless these mealybugs belonged to an "inactive" race. But this question clearly requires further investigation.

Transmission from Symptom-Free Sources.

As already stated, most transmission experiments have been made by mealybugs that had fed on symptom-bearing flush leaves of the source plant, and some on pods showing red-mottle. The following few tests have, however, been made to find out if the mealybugs can pick up the virus from parts of an infected plant showing no visible symptoms. (In all these the mealybugs used were *P. citri*.)

(a) Flush leaves that showed symptoms, but from which all symptom-bearing areas had been removed. Two transmissions out of 26 tests, one with five and the other with four mealybugs.

TABLE VI.

			Number of vectors (all instars) per test bean					
			1	2	3	4	5	6-10
<i>P. citri</i>	No. of tests ...	272	87	112	99	68	88	
	Transmissions ...	39	24	28	22	26	28	
	= per cent. ...	14.3	27.6	25.0	22.2	38.3	31.8	
<i>P. brevipes</i>	No. of tests ...	6	6	6	9	10	54	
	Transmissions ...	1	3	3	3	4	23	
<i>P. sp. near brevipes</i>	No. of tests ...	21	21	26	21	13	19	
	Transmissions ...	1	3	6	5	4	5	
<i>F. virgata</i>	No. of tests ...	—	4	3	3	9	14	
	Transmissions ...	—	1	1	1	2	1	
Total—all four vectors	No. of tests ...	299	118	147	132	100	175	
	Transmissions ...	41	31	38	31	36	57	
	= per cent. ...	13.7	26.2	26.0	23.5	36.0	32.6	

(b) The stem of a young plant the leaves of which showed symptoms. One transmission out of 16 with a single mealybug, none out of four with five mealybugs.

(c) Entirely symptom-free flush leaves from a tree that showed symptoms on other leaves. One transmission out of 23 with three mealybugs.

(d) A young infected plant, three days after the complete disappearance of the first symptoms of red vein-banding. One transmission out of four, with five mealybugs. A similar plant, about three weeks after the disappearance of the first symptoms: one out of four with ten mealybugs.

Considering the ease with which transmission can be obtained by budding, the virus is presumably systemic in the tree, but it is probable that mealybugs can pick it up more readily from flush leaves showing symptoms.

Transmission of Strain B.

As already mentioned, very few tests have been made with strain B. Using *P. citri* as vector, four transmissions have been obtained out of 17 tests, with ten second-instar, ten and six third-instar nymphs and four adults respectively. The symptom of yellow vein-banding appeared after 41, 50, 55 and 91 days: in the last case red vein-banding appeared first, after 60 days.

P. sp. near brevipes has given four transmissions out of 29 tests, with four third-instar nymphs, five, four and three adults respectively. Yellow vein-banding appeared, in three of these, after 48, 51 and 77 days: in the fourth plant red vein-banding was the only symptom to show up, after 70 days; it continued to appear on subsequent flushes, without any trace of yellow vein-banding, for over seven months, when the plant was destroyed.

No transmission of strain B has been obtained with *P. brevipes*, but only 12 tests have been made, and these with only one, two or three nymphs per bean, apart from one series of 15 tests in which the mealybugs were subjected to a post-infection-feeding starvation of 23 hours.

No transmission of strain B has yet been tried with *Ferrisia virgata*.

Movement of the Vectors.

It is clear from the diagram of the spread of the virus at River Estate given by Baker and Dale (1947b) that cacao trees in contact with an already infected tree are more likely to become infected than those not in contact, although isolated infections are by no means unknown. I believe that the natural movements of *P. citri* are quite sufficient to account for this. Species of *Pseudococcus* are usually considered to be comparatively immobile insects which, after the first instar, do not move unless disturbed, but this is not so with *P. citri* on cacao in Trinidad. On any moderately infested cacao tree, actively moving individuals can nearly always be seen at any time of the day. Most of these are full-sized adults, presumably searching for a suitable place for oviposition, but second- and third-instar nymphs can also often be found wandering about on the trunk. They move both up and down the tree and have been observed crawling over the litter on the ground.

The first-instar "crawlers" are, of course, like those of most Coccids, very active; so much so that great difficulty has been encountered in the laboratory in keeping one colony of mealybugs free from intruding crawlers from another colony, even though both were kept in apparently well-fitting glass-bottomed "pill-boxes." But this only applies to crawlers before they have fed—once they have settled down to feed they are no more active than the second and third instars, and much less so than young adults. It therefore seems probable that the first instar, in spite of its activity, cannot be responsible for much of the spread of the virus.

No experiments have yet been made to find out whether the offspring of an infective mealybug, that have not themselves fed on an infected tree, are capable of carrying the virus. Congenital transmission of virus diseases is, however, very uncommon and has only been definitely proved to occur with two leaf-hoppers. Dwarf disease of rice is transmitted through the eggs of *Nephotettix apicalis* var. *cinctipes* (Uhl.) (Leach, 1940) and club-leaf virus of clover through the eggs of *Agallioptis novella* (Say) (Black, 1948).

The longest time for which the different instars can survive without feeding has not been ascertained, but first- and second-instar nymphs can certainly survive for at least 48 hours, the third instar for three days, and young adults for five days. All stages can therefore live without feeding for considerably more than the longest time, about 23 hours, for which they are so far known to be capable of retaining the virus (p. 112).

The problem of how comparatively isolated infections arise is more difficult: infective mealybugs may perhaps be occasionally carried by birds or other animals, or the earlier instars may sometimes get dispersed by wind. But one of the main causes may be the practice, during the harvest, of making dumps of ripe pods, usually under a cacao tree, before splitting them to remove the beans. In this way numbers of mealybug colonies on pods are moved about within a plantation. Some of these will have come from infected trees and some of the mealybugs may then crawl on to healthy trees at a considerable distance from the source of infection.

From such observations as I have so far made it seems that ants do not play any appreciable part in spreading *P. citri*. On a few occasions ants have been seen to pick up a mealybug, but apparently only when the ants had first been disturbed.

Very few observations have been made on the mobility of the other three vectors. *P. brevipes* always appears to live under a covering "tent" built by ants and only in the crawler stage has this species been seen to move of its own volition. It is suspected that new colonies are founded by ants, but no proof of this has been obtained.

I have only kept a single colony of *P. sp.* near *brevipes* under fairly constant observation. Although it was a large colony, infesting several of the pods on one

tree, the species only spread to one out of four trees that were in contact with the one harbouring the original colony. This species is certainly not as active as *P. citri*.

No information is available about the movements of *Ferrisia virgata*, though it might be expected that the long glassy wax filaments of this species would render it liable to be carried long distances by the wind.

These three species, however, must be of minor importance compared with *P. citri* as regards the spread of the virus, because of their comparative rarity on cacao.

Summary.

Two strains of a virus disease, "A" and "B," which possibly may be two distinct viruses, occur on cacao in Trinidad. These are compared with the more virulent "swollen-shoot" and related viruses that are widespread in West Africa.

The most important resemblance between the Trinidad and the West African viruses is that both are carried exclusively by mealybugs of the family PSEUDOCOCCIDAE. There are also points of similarity in the symptoms, which in the Trinidad virus consist mainly of a transient red vein-banding, with or without a more or less discontinuous yellow vein-flecking (which does not disappear when the leaf matures) and, on certain varieties of cacao, red-mottle on the pods. Swellings on the shoots, a conspicuous symptom of most of the strains of the West African viruses, have not been observed in Trinidad.

Four species of mealybugs are definitely known to be vectors: *Pseudococcus citri*, which is the commonest and undoubtedly responsible for most of the natural spread of the disease; *P. brevipes*; a species near *P. brevipes* but almost certainly distinct; and *Ferrisia virgata*. Certain other mealybugs have been found on cacao in Trinidad but the virus has not yet been transmitted by them.

Most transmission experiments have been made with mealybugs bred up on potato tubers and with Posnette's technique of feeding potential vectors, after infection-feeding, on cacao beans from which one of the cotyledons has been removed, so that the insects can feed on the convoluted surface of the remaining cotyledon or on the radicle. Notwithstanding some drawbacks to this method it has many advantages over using young growing plants as tests.

The "latent period" of the virus in the test plants grown from dissected beans has varied between 20 and 123 days, though the symptoms most commonly appear from 30 to 50 days after infection of the bean. The symptom of red vein-banding has appeared much more often than that of yellow vein-flecking, irrespective of the symptoms on the virus source plant. When both symptoms appear the red vein-banding almost always shows up first.

All three immature instars, and young adults, of all four vector species are probably almost equally efficient as vectors, except that there is some indication that *P. brevipes* may be slightly more efficient than the other three species.

Starvation of the mealybugs before infection-feeding does not increase their capacity to transmit, though it usually helps to make them settle and feed more readily on the source plant.

The time for which the mealybugs feed on the virus source plant has little if any effect on their capacity to transmit the virus. Mealybugs have become infective in just over one half-hour infection-feeding, though the proportion of transmissions obtained when the infection-feeding time has been between one-half and two hours is rather less than that for over two hours, probably because in the former tests some of the insects had not actually fed.

The duration of feeding on the test bean is probably also immaterial, provided of course that the mealybugs do actually feed. The shortest test-feeding time that has resulted in transmission is 90 minutes, but this included a considerable "settling" time.

Transmission may be effected whether a mealybug feeds on the cotyledon or the radicle of a test bean.

Mealybugs can still transmit the virus if they are starved after infection-feeding for a period up to 22½ hours, but no transmission has yet been obtained when the post-infection starvation has been over 23 hours. It is not yet known whether a short period of feeding after infection-feeding renders the mealybugs incapable of transmitting, but post-infection feeding for 66 hours does so.

A transmission rate of just under 14 per cent. has been obtained with single mealybugs to each test bean but with larger numbers the rate has not risen in accordance with the mathematical expectation. A possible explanation for this might be that there are "active" and "inactive" races of *P. citri*, but so far practically no evidence for this has been obtained.

Mealybugs can become infective by feeding on the symptom-free parts of flush leaves showing symptoms in other parts, on entirely symptom-free flush leaves from infected trees, on the stem of a young infected plant, and on the leaves of a young infected plant after the disappearance of the transient symptoms of red vein-banding. It appears, however, that they pick up the virus more readily from flush leaves actually showing symptoms, and these have been used as the source of the virus in most of the experiments.

Few experiments have yet been made with strain "B" of the virus, but this strain has been transmitted with *P. citri* and *P. brevipes*.

All stages of *P. citri*, but especially young adults, often wander about of their own accord and are thus capable of spreading the virus, particularly to trees actually in contact with an already infected tree. The transport within a plantation of cacao pods during harvesting is thought to be a likely cause of isolated new infections.

Acknowledgements.

I am indebted to Professor R. E. D. Baker for much help, especially in the early stages of my investigations, for dissecting cacao beans for me on many occasions, and for allowing me the use of his greenhouse and the services of his technical assistants for looking after the test plants. Also to Mr. O. J. Voelcker, Director, and the staff of the West African Cacao Research Institute, for the invaluable information on "swollen-shoot" which they gave me during a visit of some three weeks duration which I made to Tafo in August 1948. I would take this opportunity of thanking them again, not only for their assistance on technical matters, but for their well-known "West Coast" hospitality. I also wish to thank Dr. H. Lees, of the Chemistry Department of this College, for his trouble in taking the photographs reproduced in Plate II.

References.

- BAKER, R. E. D. & DALE, W. T. (1947a). Notes on a virus disease of cacao.—*Ann. appl. Biol.*, **34**, p. 60.
 BAKER, R. E. D. & DALE, W. T. (1947b). Virus diseases of cacao in Trinidad—II.—*Trop. Agriculture, Trin.*, **24**, p. 127.
 BLACK, L. M. (1948). *Phytopathology*, **38**, p. 2.
 *BLATTNÝ, C. & VUKOLOV, V. (1932). Mosaik bei *Epiphyllum truncatum*.—*Gartenbauwissenschaft*, **6**, p. 425. (*Rev. appl. Mycol.*, **12**, p. 294, 1933.)

*Abstract only consulted.

- CIFERRI, R. (1930). Phytopathological survey of Santo Domingo, 1925-1929.—J. Dep. Agric. P.R., **14**, p. 5.
- CIFERRI, R. (1948). Una virosis del cacao en Colombia y en la República Dominicana.—Rev. Fac. nac. Agron., Medellín, **8**, no. 29-30, p. 79.
- *ELMER, O. H. (1925). Transmissibility and pathological effects of the mosaic disease.—Res. Bull. Iowa agric. Exp. Sta., no. 82. (Rev. appl. Mycol., **4**, p. 754, 1925.)
- FALARDO, T. G. (1930). Studies on the mosaic disease of the bean (*Phaseolus vulgaris*).—Phytopathology, **20**, p. 469.
- FENNAH, R. G. (1947). The insect pests of food crops in the Lesser Antilles. 207 pp. St. George's, Grenada, &c., Dep. Agric. Windw. & Leew. Is.
- JAMES, H. C. (1937). Sex ratios and the status of the male in Pseudococcinae.—Bull. ent. Res., **28**, p. 429.
- LEACH, J. G. (1940). Insect transmission of plant diseases. 615 pp. New York and London, McGraw-Hill.
- OLITSKY, P. K. (1925). The transfer of tobacco and tomato mosaic disease by *Pseudococcus citri*.—Science, **62**, p. 442.
- POSNETTE, A. F. (1941). Swollen-shoot virus disease of cacao.—Trop. Agriculture, Trin., **18**, p. 87.
- POSNETTE, A. F. (1944a). The diagnosis of swollen-shoot of cacao.—*Ibid.*, **21**, p. 56.
- POSNETTE, A. F. (1944b). Virus diseases of cacao in Trinidad.—*Ibid.*, **21**, p. 105.
- POSNETTE, A. F. (1947). The use of seeds in the insect transmission of some plant viruses.—Nature, Lond., **159**, p. 500.
- POSNETTE, A. F. & STRICKLAND, A. H. (1948). Virus diseases of cacao in West Africa. III. Technique of insect transmission.—Ann. appl. Biol., **35**, p. 53.
- STOREY, H. H. (1932). The inheritance by an insect vector of the ability to transmit a plant virus.—Proc. roy. Soc., (B) **112**, p. 46.
- VOELCKER, O. J. (1946). West African Cacao Research Institute, Tafo. Annual Report 1944-45.
- VOELCKER, O. J. (1948). West African Cacao Research Institute, Tafo. Annual Report 1946-47.
- WATSON, M. A. (1946). The transmission of beet mosaic and beet yellows viruses by aphides.—Proc. roy. Soc., (B) **133**, p. 200.
- WATSON, M. A. & ROBERTS, F. M. (1939). A comparative study of the transmission of *Hyoscyamus* virus 3, potato virus Y and cucumber virus 1.—*Ibid.*, **127**, p. 543.
- WOLCOTT, G. N. (1933). An economic entomology of the West Indies. 688 pp. San Juan, P.R., Ent. Soc. P. Rico.



FIG. 1. Young leaf with narrow red vein-banding restricted to certain lengths of second- and third-order veins.



FIG. 2. Young leaf with broad red vein-banding covering most of lamina.

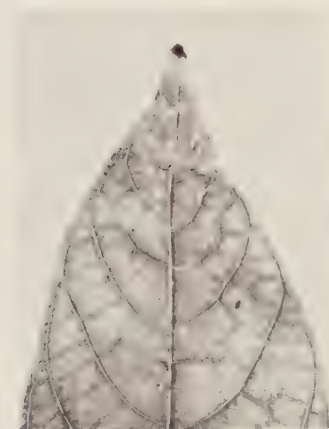


FIG. 3. Young leaf with fairly general conspicuous red vein-banding mainly on second- and some third-order veins; also large isolated chlorotic vein flecks near tip.



FIG. 4. Young leaf with chlorotic vein-flecking and short lengths of red vein-banding.



FIG. 5. Leaf with chlorotic vein-flecking, mainly on midrib and second-order veins, becoming continuous vein-banding towards tip of leaf.



FIG. 6. Mature leaf with conspicuous chlorotic banding of midrib, second-order veins and basal parts of some third-order veins.

THE COCCIDS OF CACAO IN BAHIA, BRAZIL.

By Pedrito SILVA.

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Coccids on cacao have received much attention in recent years in connection with the transmission of swollen-shoot disease in the West African cacao belt and many papers on them have been published by British entomologists.

Strickland (1947) listed 17 species of Coccids "from cacao in parts of the world other than West and Central Africa and San Thomé . . .", but he did not include records from Bahia. In view of this omission and the difficulty of obtaining references to Brazilian literature,* I have decided to summarise the available information on Coccids occurring on cacao in Bahia.

Zehntner (1914) was the first to report a Coccid on cacao in Bahia: the insect observed by him attacked the pods and young shoots and was recorded under the name *Dactylopius*. Bahiana (1917) reported some unidentified Coccids on cacao from the same State. Bondar (1922), dealing with some insect pests of cacao in Bahia, drew attention to the trophobiosis that occurs between certain unidentified Coccids and the ant, *Azteca chartifex* var. *spiriti* For., and also described the "sheds" constructed over them by this ant on twigs, pods and fruit-stalks. Surveying the insect pests of the principal crops grown in Bahia, Azevedo (1923) reported *Pseudonidia trilobitiformis* (Green) and *Coccus viridis* (Green) on cacao. Bondar (1925), in a second contribution on insect pests of cacao in Bahia, described four new species of Coccids, viz., *Ceroplastodes bahiensis*, *C. costa-limae*, *C. melzeri* and *C. theobromae*, all of them in trophobiosis with *Azteca chartifex* var. *spiriti*, and recorded *Saissetia hirsuta* (Newst.), *Coccus viridis*, *Aspidiotus destructor* Sign., *A. cyanophylli* Sign. and *Pseudonidia trilobitiformis*. Azevedo (1929), in another survey of the insect pests of the major crops of Bahia, reported *Aspidiotus destructor*, *A. cyanophylli*, *Ceroplastodes bahiensis*, *C. theobromae*, *Coccus viridis* and *Pseudonidia trilobitiformis* on cacao. Moreira (1929) reported Coccids of the genera *Aspidiotus*, *Ceroplastodes*, *Coccus* and *Pseudonidia* damaging cacao in Bahia, and Lepage (1938) listed the following species attacking cacao: *Aspidiotus cyanophylli*, *A. destructor*, *Ceroplastodes bahiensis*, *C. costalimai* Bondar (presumably a mis-spelling of *costa-limae*), *C. melzeri* Bondar, *C. theobromae*, *Coccus viridis*, *Pseudonidia trilobitiformis* and *Saissetia hirsuta* Bondar (1939) again reported the same species as in 1925, but added observations on *Pseudococcus citri* (Risso) and its trophobiotic association with ants of the genus *Solenopsis*. Silva (1944) gave a general account of the insect pests of cacao in the State of Bahia, citing the Coccids already reported as attacking cacao there and the ants associated with them, and giving also the results of work on mealybug control.

Dr. Walter Carter, of the *Pineapple Research Institute* of Hawaii, visited me in October, 1946, the principal aim of his visit being to investigate the distribution of *Pseudococcus brevipes* (Ckll.), its parasites and the "green spotting of pineapple" in south-east Bahia. We collected *P. citri* in trophobiosis with ants on the leaves, flower buds, young shoots and fruit of cacao. In a forthcoming paper, Dr. Carter will discuss the results, chiefly the identity of the species other than *P. citri* collected on cacao trees belonging to the Trinitario group, which shows virus-like symptoms.

* To remedy this deficiency, the writer is preparing "Bibliografia sobre os insetos associados ao cacauero, cacau e chocolate no Brasil", which will be printed very shortly.

There is some doubt as to the identity of some of the Coccids attacking cacao in Bahia and I am taking the necessary steps for them to be carefully checked. Dr. Helio Lepage, an outstanding Brazilian coccidologist on the staff of the Biological Institute at São Paulo, is studying the species of the genus *Ceroplastodes* that were described by Bondar (1925); Dr. Carter is dealing with species of the genus *Pseudococcus*.

References.

- AZEVEDO, A. (1923). *Correio agric.*, **1**, pp. 152.
AZEVEDO, A. (1929). *Ibid.*, **7**, p. 85.
BAHIANA, J. (1917). *Bol. Sec. Agric. Bahia*, No. 3, p. 69.
BONDAR, G. (1922). Cacao: a cultura e as pragas do cacoeiro no Estado da Bahia, Brasil, pp. 41-43, fig. 22. *Bahia, Sec. Agric.*
BONDAR, G. (1925). O cacao, pt. II: Molestias e inimigos do cacoeiro na Estado da Bahia, Brasil, pp. 55-63, figs. 36-37, 39. *Bahia, Sec. Agric.*
BONDAR, G. (1939). Insetos daninhos e parasitas do cacau na Bahia.—*Bol. téc. Inst. Cacau Bahia*, no. 5, pp. 36-44, 104, figs. 20, 57.
LEPAGE, H. (1938). Catalogo dos Coccideos do Brasil (Homoptera—Coccoidea).—*Rev. Mus. paulista*, **23**, pp. 348-349, 351, 370, 393-394, 418.
MOREIRA, C. (1929). Entomologia agricola brasileira.—*Bol. Inst. biol. Def. agric.*, Rio de J., no. 1 revd., 174 pp. [not seen].
SILVA, P. (1944). *Trop. Agriculture, Trin.*, **21**, pp. 11-12.
STRICKLAND, A. H. (1947). *Bull. ent. Res.*, **38**, p. 521.
ZEHNTER, L. (1914). *Le cacaoyer dans l'Etat de Bahia*, p. 122. Berlin.
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THE WETTING OF INSECT CUTICLE.*

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(Plate III.)

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1. PHYSICAL FACTORS.

The wetting of insect cuticle by liquids has received little attention in spite of the fact that the effectiveness of almost all contact insecticides depends upon it. Although a considerable amount of work has been done in the past few years upon the mechanism of wetting and spreading of liquids on solids, Stellwaag (1924), English (1928), Wilcoxon and Hartzell (1931) and O'Kane & others (1932) are the only workers who have studied the wetting of insects.

The object of the present investigation was to study the behaviour of spray droplets when applied to the integument of insects and to make quantitative determinations of the wetting and spreading power of these liquids.

Phenomenon of Wetting.

Hamilton (1930) has suggested that "the wetting of a solid by a liquid occurs when the liquid comes into direct contact with the solid, so that no layer of air exists between the two materials . . . spreading occurs when the liquid spreads or creeps over the surface of the solid so that it covers an area greater than that which it covered when first placed upon the solid". These definitions seem reasonably explicit.

Wetting and spreading are connected with the angle which the surface of the liquid makes with the solid, i.e. the contact angle. If the contact angle is zero, the wetting is complete or perfect : if the contact angle is finite, wetting is incomplete or imperfect.

* This paper represents part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

The mathematical basis for the angle of contact as a measure of wetting lies in the expression :—

$$Y_{sa} = Y_{sl} + Y_{la} \cos \theta$$

in which Y_{sa} , Y_{sl} and Y_{la} are the solid/air, solid/liquid and liquid/air tensions respectively, and θ is the angle of contact of the liquid upon the solid. Thus it will be seen that the measurements of this angle take into account all the three forces acting upon a drop on a solid surface (fig. 1).

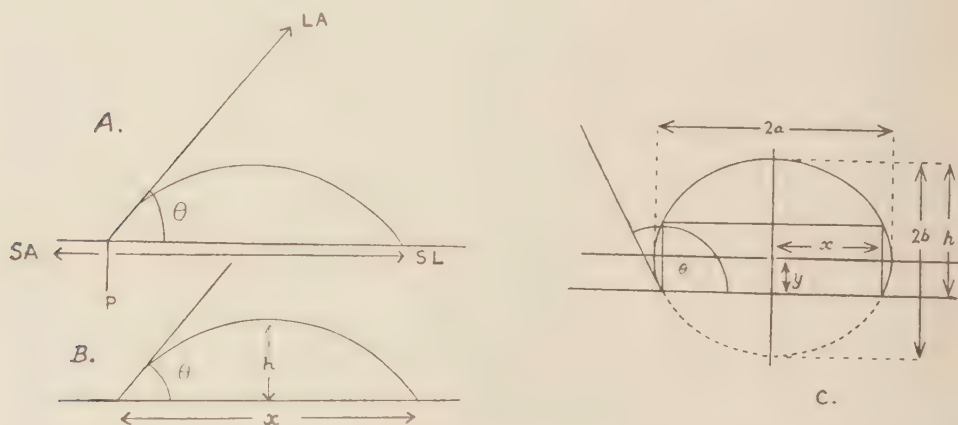


Fig. 1.—A, diagram of the forces acting on a drop of liquid on a solid surface. B, illustrating the calculations of contact angle from drop outlines; for drops less than 90° . C, for drops of contact angle greater than 90° (after Fogg) 1947.

When a drop of liquid wets a surface, it spreads and for a period gives an "advancing" angle. If sufficient liquid is withdrawn from the drop, so that it recedes over the previously wetted part of the surface, a "receding" angle is obtained, which is always smaller than the advancing angle. But, in the study of insecticides, the advancing angle may be affected by dynamic conditions, according to whether a drop spreads gradually over a surface or impinges upon it. What little work has been done, is based on measurements of contact angles at rest on a solid surface; but the measurements of angles of contact under static conditions are not an accurate criterion of the wetting and spreading ability of a liquid under these dynamic conditions.

During the present investigation a new concept of contact angles under "static" and "dynamic" conditions has been put forward and an attempt has been made to measure the contact angles under dynamic conditions.

Method.

There are various methods of measuring the contact angles formed by a liquid upon a solid (Bartell, 1941). The sessile drop method, in which drops of liquid in contact with a surface may be continuously observed, has been found suitable in biological work.* Besides being a direct method, it has the advantage of giving a value for the contact angle which is the integral of the sum of all the separate angles along the circumference of the drop (Mack, 1936). Based on this principle, methods have been devised either of photographing or tracing the outline of a drop in contact with solid surface (O'Kane & others, 1930; Ebeling, 1939).

* With twigs and leaf surfaces, Stellwaag (1924) and English (1928) have used the "plate method" for contact angle determinations.

A similar apparatus for this purpose has been improvised (fig. 2). The values of contact angles are computed from the characteristics of the curve obtained by photographing or tracing the projected profile of drops (Pl. III, figs. 1-3). For measuring the advancing contact angles under dynamic conditions, the drops were either allowed to impact on the test surface from a spray or to advance over the surface while being continuously fed by a fine capillary tube (0.06 mm. bore).

A microburette, as described by Lane (1937) for producing small liquid drops of known size, was used to measure the angles produced by impacting droplets. The drops are formed at the tip of a hypodermic needle, and a concentric stream of air blows off the drops. The whole apparatus was arranged so that the droplets fell to the test surface placed in the microprojector apparatus.

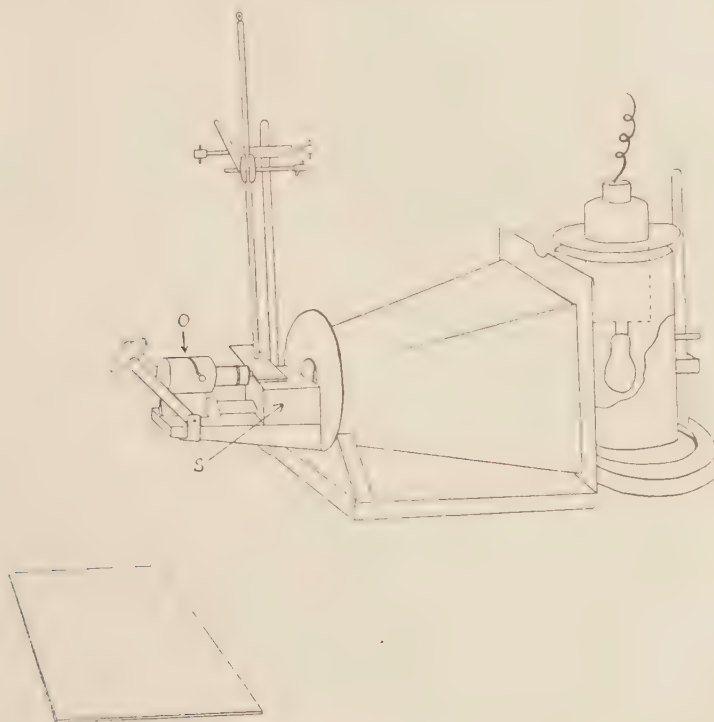


Fig. 2.—Microprojector apparatus for contact angle measurements.
O=objective, S=stage.

The insect is mounted horizontally and anaesthetised immediately before the operation by ammonia vapour; chloroform and ether were avoided because of their effect on cuticular lipoids.

In calculating values for contact angles, drops are assumed to be spherical for angles up to 90° . For angles more than 90° the figure of the drop approximates to an ellipse. In the former case Mack's formula was used. It states that:—

$$\theta = 2 \tan^{-1} (h/x)$$

Where h is the greatest height of the drop and x is the radius of the base of the drop.

In the latter case the equation for an ellipse:—

$$\theta = 180 - \tan^{-1} (x/y) \cdot b^2/a^2$$

may be used where x and y are co-ordinates of the point where the surface cuts the ellipse and a and b are the major and minor semiaxes respectively (Fogg, 1947) (fig. 1).

The method was tested by placing drops of different liquids on a standard beeswax surface and measuring the advancing contact angle. This was prepared by dipping glass slides or cover glasses into melted beeswax, or beeswax dissolved in carbon tetrachloride and allowing the wax to solidify under pressure at room temperature. If the pressure is not applied while cooling, the wax solidifies with an uneven surface. It was possible in this manner to obtain a fairly reproducible standardised smooth surface. As the drops were not of constant size, considerable variations were observed. The experiment was repeated by placing drops of distilled water and odourless distillate (diameter from 1 to 5 mm. at the base) on

TABLE I.

Contact angle on beeswax surface of fine drops of distilled water and odourless distillate in each of five drops dimensions.

Experiment	Width of the drop in mm. at the base	Mean Contact Angle (5 replicates)	
		Distilled water	Odourless distillate
1	1	101	23
2	2	97	23
3	3	95	21
4	4	94	19
5	5	94	20

	Analysis of Variance							
	Sums of squares	Degree of freedom	Variance	Variance ratio	Sums of squares	Degree of freedom	Variance	Variance ratio
A	20.6544	4	5.1636	10.095 P .001	1.4656	4	.3664	4.21 .05 P .01
B	216.2944	4	54.0736		49.5096	4	12.3774	
Residual error	85.7016	16	5.3563		47.0384	16	2.9399	
Total	322.6504				98.0136			

Differences between (A) drops of same size, (B) drops of different size.

beeswax surface. Five measurements were made of drops of each size and the contact angles computed by Mack's formula (Table I). It was found that the drops of the same size gave reproducible values. Tested by the analysis of variance, no significant difference in contact angle was found within drops of one size.

Insect cuticle is a most difficult surface to study since, besides being covered with cuticular lipoids and cement, etc., the surface of an insect, even in a limited area, usually exhibits irregular or compound curvatures, minute irregularities, hairs and scales. Contact angles may vary greatly from place to place on the same insect and apparently comparable insects may give different values. Average values have, therefore, been used for valid comparisons.

Factors affecting the Measurement of Contact Angles.

Table II summarises the different values of contact angles obtained for various liquids on a beeswax surface under dynamic and static conditions; the two are

significantly different. The static angles are much lower than the advancing angles and fall between the advancing and receding angle. Moreover, static angles are not an accurate criterion of the wetting and spreading ability of a liquid under the dynamic conditions existing when a spray is being applied. For true appreciation of the wetting and spreading ability of a liquid, therefore, contact angle values should be obtained under dynamic conditions. There is practically no correlation as pointed out by Ben-Amotz and Hoskins (1937) between dynamic and static conditions and results obtained in the latter case give no reliable information as to the behaviour of a spray in actual use.

TABLE II.

Contact angle measurements under dynamic and static conditions.

		Distilled Water and Beeswax surface		P31 and Beeswax surface		Methylphthalate and Beeswax surface	
		No. of Observations	Mean	No. of Observations	Mean	No. of Observations	Mean
Dynamic*	...	5	98° 38'	5	40° 5'	5	64° 26'
Static	...	5	84° 14'	5	25° 56'	5	55° 38'
Difference	...		14° 24'		14° 9'		8° 48'
P01		.01		.01

* Contact of angle of droplets spreading without force of impact.

In the study of insecticides, therefore, the *dynamic* contact angles of impinging droplets as opposed to *static* contact angles of drops involving no kinetic energy have to be considered.

Dynamic conditions.

A careful study was made of the effect of "impact force" on the wetting and spreading of spray droplets. For this purpose, a special technique had to be developed in order to obtain individual spray droplets of known size, to measure the contact angle at the moment of impact of drops and to measure the velocity of these droplets.

The equipment for obtaining small droplets of known size has been described above. For measuring the velocity of droplets, an apparatus (fig. 3), adopted with slight modifications from the one used by Schmidt (1909) for direct determination of the velocity of falling rain drops, was used consisting of two cardboard discs (5.25 cm. radius) fixed concentrically on a vertical axis at a distance of 8 cm. The upper disc had a sector cut in it and a scale showing degrees was marked on the lower disc. An electric motor rotated the whole apparatus about its vertical axis at a uniform speed of 200 r.p.m.

The apparatus is placed under the spray and some spray droplets reach the lower disc through the slit in the disc above. The relative position of the spray droplets on the lower disc was recorded and the velocity of the droplets was computed from the angle through which the lower disc had rotated while they traversed the distance between the two discs.

The sizes of droplets were checked by catching them on a glass slide smeared with a mixture of oil and vaseline and measuring them directly under the microscope, assuming them to be spherical. The droplet size may also be measured indirectly if the droplets are allowed to fall on a filter paper dusted with eosin fixed on to the lower disc in the apparatus described above. The blot produced by the drop on

the filter paper will be according to the size of the spray droplet. The method can be calibrated to give fairly accurate values.

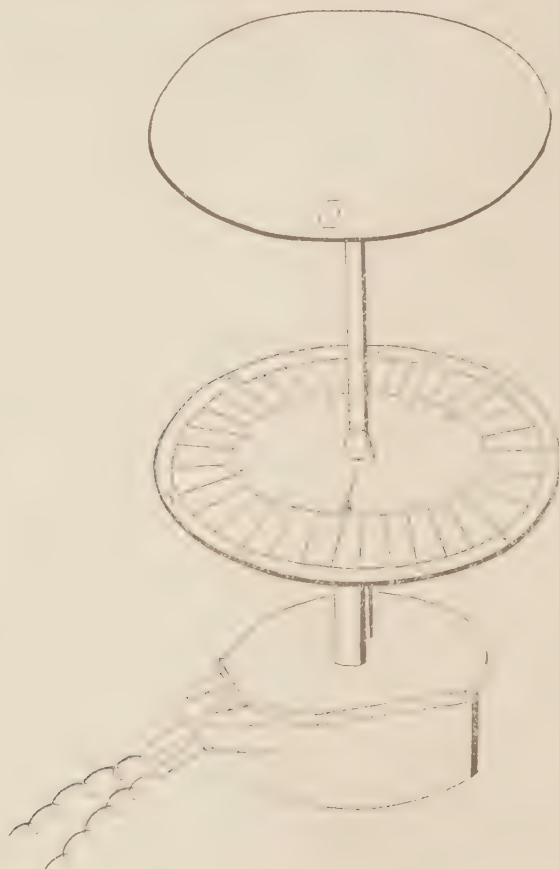


Fig. 3.—Apparatus for measuring the velocity of droplets.

The effect of impact force on wetting and spreading was studied by allowing droplets of distilled water to impact on a standard beeswax surface. Contact angle measurements were made immediately after impact and the forces of impact calculated by the formula $\frac{1}{2} mv^2$. The following results were obtained :—

					Advancing Contact Angle		
					Drops involving no force of impact	Coarse spray	Fine spray
						(Contact angles of impinging droplets)	
(i) Contact angle (in degrees)	101	100	101
(ii) Average drop size (diameter)	·3 mm.	·5 mm.	·1 mm.
(iii) Force of impact	0	(1·3–3·3 ergs)	(0·00019–0·035 ergs)

From these figures it is evident that the contact angle of drops used in insecticidal spray (0.1 to 0.5 mm.) is not influenced by force of impact.

When, however, drops of distilled water of larger size (5 mm. diameter) were allowed to fall from a capillary tube on to a standard beeswax from heights varying from 5 to 20 cm. there was a slight reduction in the magnitude of advancing contact angles.

		Average contact angle in degrees
Advancing contact angle of drops involving no kinetic energy ...	—	101
Drops allowed to impact from a height of	5 cm.	95
	10 cm.	94
	15 cm.	95
	20 cm.	95

In the spraying of plants or other surfaces where coarser sprays are used, this phenomenon may be of considerable practical value. If "adhesives" are incorporated in sprays, not only will each drop cover a larger area, but it will be retained and increase the total amount of spray deposit.

Static conditions.

Size of the drop.—Drops of distilled water, odourless distillate and methyl salicylate, which ranged in diameter from 1 to 5 mm. at the base, were placed on beeswax surface. Five measurements were made of each drop dimension and the contact angles computed from the data by Mach's formula (Table I). It was found that drops more than 3 mm. gave slightly lower values of contact angles with both aqueous and oil solutions. Probably this is due to the effect of gravity flattening larger drops. Ebeling (1939), however, working on leaf surfaces, found no such

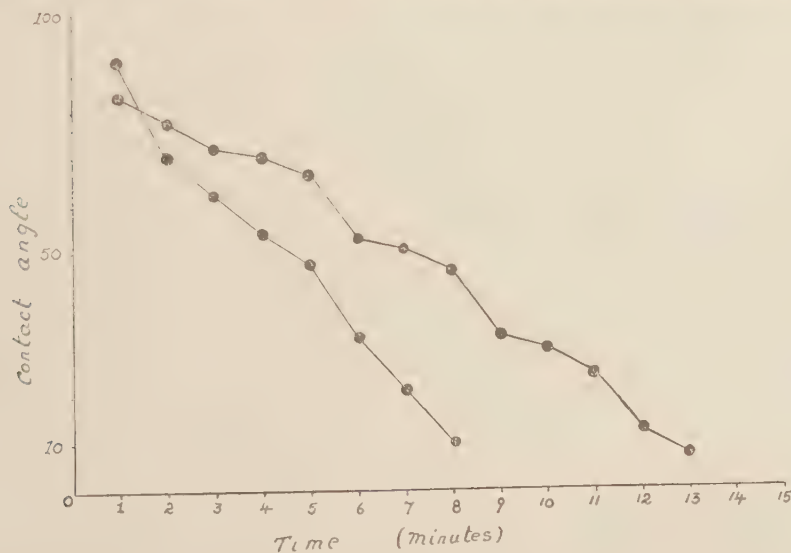


Fig. 4.—Showing consecutive determinations of contact angles—distilled water on beeswax.

discrepancy, though in confirmation of his observations a drop diameter of about 3 mm. was found to be the most convenient size for manipulation and for repeated comparative observations.

Time interval.—The first series of static contact angle measurements was made without taking into consideration the time interval between placing the drop and tracing its image. The results obtained were erratic and unsatisfactory. These experiments were repeated, making consecutive determinations of contact angles of drops at intervals of one minute. It was found that the contact angle of distilled water progressively decreased with the interval of time from 0 to 15 minutes, whereas with oily liquids (odourless distillate and methyl phthalate), after a slight decrease in the first three to four minutes, the contact angles remained steady for quite a long time (observations recorded up to 24 hours later) (figs. 4 and 5). This is presumably due to the evaporation of the water and consequent decrease in its bulk leading to a change from an advancing to a receding angle.

Most solids and liquids show this phenomenon called "hysteresis" of the contact angles, that is the difference between the advancing and the receding angles of contact. Water gives a high value of hysteresis, often 60° or more, the liquid being able to rest on the solid at any angle between the two extremes of the advancing and the receding angle (Adam, 1941).

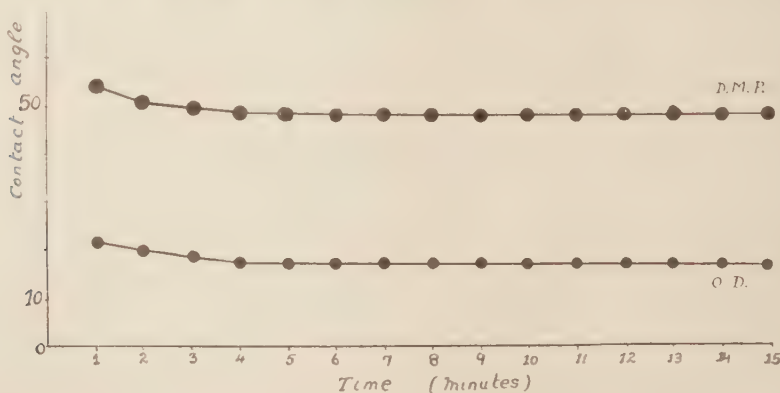


Fig. 5.—Showing consecutive determinations of contact angles—dimethyl phthalate and odourless distillate on beeswax. (D.M.P. and O.D.)

Considerable variations in temperature and humidity have a very slight effect on contact angle. These factors are unlikely to affect evaporation of water drops during the time necessary to trace the image as it takes only a fraction of a second to trace the drop outline. Moreover, evaporation of spray oils is even slower. No attempt was made, therefore, to control the temperature and humidity conditions in the working room, where these factors were fairly constant. The temperature measurements were, however, recorded by suspending a thermometer close to the horizontal platform of the microprojector.

2. BIOLOGICAL FACTORS.

Variations in different insects.

The contact angles made by 17 different liquids on 30 species of insects were investigated. An endeavour was made to include insects differing widely in the nature of their integument and habitat. For comparison, corresponding measurements were made on beeswax surface.

Several species representing insects from different habitats such as terrestrial or aquatic (both submerged and surface skaters), subterranean (both under wet and dry conditions) and insects of agricultural and medical importance, have been studied. These insects, also, represented a wide variation in the nature of their integument from the smooth surface of cockroach and *Tenebrio* to the more complicated surface of hairy and scaly insects such as flies, mosquitos and caterpillars, etc.

The test liquids* included saturated and unsaturated hydrocarbons, hydrocarbons with varying aromatic contents, spray oil, organic esters, alcohols and aqueous solutions. Specifications of oils are give in Table III.

TABLE III.
Specifications of oils.

	S.G.	Boiling Point (°C.)		Viscosity Redwood I (secs. at 21°C.)	Un- sulphonat- able residue
		IBP	FBP		
Odourless distillate	·779	198	257	32	Per cent. 99
White Oil P31	·857	300	(75 per cent. at 367)	140	99
A.I.O.C. Spray Oil	·848	324	395	110	
Shell Mex A12	·825	265	365	44	86
Shell Mex KB30	·865	310	380	95	91
Kerosene (High aromatic) ...	·845	180	260	—	62
Kerosene (Low aromatic) ...	·789	168	294	—	91
Kerosene (Non-aromatic) ...	·789	203	261	—	99·5
White Spirit	·794	150	200	—	77

Preliminary experiments were carried out by applying minute droplets of liquids (0·03 mm. diameter) by means of a micropipette to the integument of insects and observing their wetting and spreading ability. The behaviour of droplets applied as a spray was also studied. Distilled water, methyl phthalate, white oil P31, spray oil and odourless distillate were selected amongst the liquids for detailed quantitative studies. Mean contact angles on 17 species of insects are given in Table IV.

As will be seen from Table IV, the relative wetting properties of a liquid varied greatly with different species of insects. For example, distilled water formed an angle of approximately 180° on mosquitos, 110° on *Tribolium*, 18° on cabbage moth larvae. Methyl phthalate formed an angle of 67° on *Tenebrio*, 55° on *Rhodnius*, 45-50° on *Ornithodorus* and 35° on *Periplaneta*. P31 formed an angle of 30° on *Rhodnius*, 22° on *Tenebrio* and approximately 0° on most other insects. Spray oil and odourless distillate formed an angle of approximately 25-28° on *Tenebrio* and beeswax surface, but completely wetted all other insects.

Variations in different parts of the body.

Table V gives results of contact angle determinations on different parts of the body of several species and it was observed that contact angles were generally lower on the ventral than on the dorsal side in most insects. Distilled water formed an angle of 83° and 93° respectively on the ventral and dorsal surfaces of *Tenebrio* larvae, 75° and 83° respectively on the ventral and dorsal surfaces of *Periplaneta* and 77° and 95° respectively on the ventral and dorsal surfaces of *Rhodnius*. With oily liquids, however, the variations in contact angles on the dorsal and ventral surfaces were not specific and constant.

* Odourless distillate, kerosene non-aromatic, kerosene low-aromatic, kerosene high-aromatic, white oil P31, A.I.O.C. spray oil, Shell KB30, Shell A12, Malariol, oil of wintergreen, dimethyl phthalate, terpineol, methyl alcohol, ethyl alcohol and distilled water.

TABLE IV.

Advancing contact angle in degrees of various liquids when applied to the integument of insects.

Insects	Stage	Liquids				
		Distilled water	Methyl phthalate	White oil P31	Spray oil	Odourless distillate
1. Beeswax surface for comparison		97°	63°	39°	33°	23°
2. <i>Periplaneta americana</i> (L.)	Adult	83°	35°	0 approx.°	0° approx.	0° approx.
3. <i>Rhodnius prolixus</i> Stål	Adult	95°	55°	30°	0°	0°
4. <i>Triatoma infestans</i> (Klug)	Adult	105°	60°	28°	0°	0°
5. <i>Naucoris cimicoides</i> (L.)	Adult	110°	41°	0°	0°	0°
6. <i>Notonecta glauca</i> L.	Adult	80°	98°	0°	0°	0°
7. <i>Aphis</i> sp.	Adult	approx. 62°	44°	0°	0°	0°
8. <i>Macrosiphum</i> sp.	Adult	106°	56°	0°	0°	0°
9. <i>Arctia caja</i> (L.)	Larvae	180°	46°	0°	0°	0°
10. <i>Mamestra brassicae</i> (L.) and <i>Polia oleracea</i> (L.)	Larvae	18°	50°	27°	0°	0°
11. <i>Tenebrio molitor</i> L.	Larvae	93°	67°	22°	27°	23°
12. <i>Tribolium confusum</i> Duv.	Adult	110°	56°	22°	0°	0°
13. <i>Calandra granaria</i> (L.)	Adult	107°	54°	26°	0°	0°
14. <i>Ephestia kühniella</i> Zell.	Adult	85°	36°	0°	0°	0°
15. <i>Culex pipiens</i> L.	Adult	180°	43°-17°	0°	0°	0°
16. <i>Musca domestica</i> L.	Larvae	48°-65°	36°-43°	0°	0°	0°
17. <i>Ornithodoros moubata</i> (Murr.)	Adult	43°-95°	45°-50°	0°	0°	0°

All readings taken on the dorsal surface.

It was also found that the liquids spread more readily upon the legs and antennae of Diptera, Orthoptera and Hemiptera than on the wings and other parts of the body. This is of considerable interest because these observations suggest that spray liquids most easily contaminate the legs which are assumed to be the point of entry of residual film insecticides.

Tests were therefore made to study the distribution of spray droplets following an exposure to a spray mist in order to ascertain on which parts of the body of an insect droplets generally accumulate. David (1946a) measured the quantity of spray accumulated by individual flies and mosquitos on the body and wings, using dyed oil and colorimetric estimation to compare the quantity of spray accumulated on the body with that on the appendages. In the present work, oils containing fluorescent powders were used and the spray droplets detected by their fluorescence under the ultraviolet radiations.*

When motionless insects are sprayed with odourless distillate, droplets are spread over all parts of the body and most of the droplets cling to scales and hairs. But, when insects are allowed to fly through a spray, it is found that while droplets are distributed at random over the whole body, a relatively larger accumulation is found on the wings. Likewise aqueous sprays do not generally wet the integument but accumulate on antennae, mouthparts, hairs and scales, etc. (Pl. III, figs. 4 and 5). Since this problem is of great practical importance, a more detailed investigation has been made of the wetting of these structure, the results of which are recorded later.

* The fluorescent compounds used were anthracene and photophour giving bright green and blue fluorescence. The ultraviolet lamp used was 125 watts "Mercra" lamp with a concentrating reflector. I should like to thank the British Thomson-Houston Co., Ltd., for the loan of apparatus, for supplying fluorescent compounds and for other technical assistance.

TABLE V.

Advancing contact angle in degrees of various liquids when applied to the integument of insects.

Insects	Stage	Region	Liquids		
			Distilled water	Methyl phthalate	White oil P31
<i>Periplaneta americana</i> ...	Adult	Dorsal ...	83°	35°	0°
		Ventral ...	75°	34°	0°
		Legs ...	63°	26°	0°
		Antennae	65°	29°	0°
<i>Rhodnius prolixus</i>	Adult	Dorsal ...	95°	55°	30°
		Ventral ...	77°	47°	18°
		Legs ...	67°	32°	17°
		Wings ...	85°	36°	0°
<i>Triatoma infestans</i>	Adult	Dorsal ...	105°	60°	28°
		Ventral ...	73°	53°	22°
		Legs ...	70°	37°	17°
		Wings ...	97°	39°	0°
<i>Notonecta glauca</i>	Adult	Dorsal ...	180°	98°	0°
		Wings ...	30°	60°	0°
<i>Tenebrio molitor</i>	Larva	Dorsal ...	93°	67°	22°
		Ventral ...	83°	67°	19°
<i>Tribolium confusum</i>	Adult	Dorsal ...	109°	56°	22°
		Ventral ...	77°	47°	18°
		Legs ...	73°	41°	17°
<i>Culex pipiens</i>	Adult	Dorsal ...	180°	43°	0°
		Ventral ...	180°	39°	0°
		Legs ...	75°	37°	0°
		Wings ...	95°	41°	0°
<i>Ornithodoros moubata</i> ...		Dorsal ...	43°-49°	45°-50°	0°
		Ventral ...	39°-43°	33°-38°	0°

Causes of Variations in the Magnitude of Contact Angles.

Nature of cuticle.

The cuticle of insects is covered by lipoids consisting chiefly of hydrocarbons and esters. The pure extracted cuticular lipoids are, according to Beament (1945) solid waxes (except in Blattids which have a mobile grease) and form a layer approximately 0.25 μ thick on the epicuticle of most insects. The waxes show a gradation of physical properties corresponding to their melting points and at this temperature undergo a crystalline transition; the molecules become mobile, and the layer is disorganised.

Cement or other deposits of dried secretions from glands of the integument also affect the wetting of insects and great differences may, therefore, be expected to occur among various species.

In general, most insects are readily wetted by oils, but are unwetted by water. The cabbage and tomato moth larvae (*Mamestra* and *Polia*) and a few other Muscid larvae (Table IV) are both lipophilic and hydrophilic. The wetting of moth larvae by water could be explained by the presence of secretory contaminations covering the lipoids on the integument of these insects. The integument of larvae, which live under extremely dry conditions, is, however, devoid of secretory contaminations.

One could postulate the absence of lipoid covering on the epicuticle of such larvae or perhaps the sand particles in which these larvae were reared cause abrasions on the wax layer.

From the data available it seems that hard cuticular lipoids, as those of *Calandra* and *Tribolium*, are more strongly hydrophobic (angle of contact $107-110^\circ$) than are the greasy type of waxes of Blattids (contact angle 83°).

Contact angle measurements were also made on the integument of insects after washing with ether or chloroform in order to dissolve away the cuticular lipoids, but no significant difference was noted in the contact angle. After extraction for a few hours (3-6 hours at room temperature), however, the integument was rendered hydrophilic.

Ageing of the cuticle.

The chemical nature of the outer layer of the cuticle shows marked changes during the period of moulting. As Wigglesworth showed recently (1947), the newly moulted cuticle of *Rhodnius* is completely hydrophobic; but it undergoes a change in the first two hours when it shows hydrophilic properties and finally it becomes persistently hydrophobic again after four hours. Some measurements confirm this, as follows :—

Period after moulting	Contact angle	Remarks
Immediately...	110°	Hydrophobic.
One hour ...	90°	Droplets adhere to surface.
Two hours ...	85°	
Three hours ...		Droplets still adhere to surface.
Five hours ...	95°	Cuticle fully darkened and hydrophobic again.

Physical structure.

The extent to which a solid is wetted by a liquid depends not only on the attraction which exists between the molecules of the liquid and the chemical groups exposed in the solid surface but also on physical structure of the surface (Adam, 1941). It has also been shown that the hydrophobic property of aquatic birds is largely due to the physical texture of the feathers and not to the presence of any special oil or wax as is commonly believed (Cassic & Baxter, 1945). The same is true of animal furs and cabbage leaves.

Interesting examples of the effect of the structure of a surface on the contact angle are shown by different insects (Tables IV and V). When a surface is easily wetted by a liquid, increased roughness has the effect of lowering the contact angle; when, however, the liquid does not easily wet the solid, roughness has the opposite effect. For example, odourless distillate spreads more readily on hairy than on smooth insects, whereas water spreads comparatively better on smooth than on hairy insects. When irregularities are present, the solid surface under the drop is separated from the water at various places by a film of air, and contact angles determined under such conditions are higher than the true contact angles. Wenzel (1936) and Cassic & Baxter (1945) have studied this phenomenon in detail and have shown on theoretical grounds that the contact angle on a discontinuous surface (θ_D) is related to the normal contact angle by the following formula :—

$$\cos \theta_D = f_1 \cos \theta - f_2$$

Where f_1 is the area of solid-liquid contact and f_2 is the area of the interface. From this equation it follows that when the contact angle is *above* 90° (poor wetting) the apparent contact angle is *increased*; and vice versa.

Furthermore, the effect of a discontinuous surface is related to the relative sizes of the areas of contact and air gaps, there being an optimum ratio between the two. In a duck's feather, or the plastron of certain water bugs, the minute filaments are rigidly fixed at this optimum distance apart. In other insects the hairs are usually freely moveable distally, with the result that when a drop of water, or other liquid, falls on to them, the tips of the hairs are drawn together, due to surface tension (Pl. III, figs. 4 and 5). Nevertheless, the bases of the hairs are held at a fixed distance and thus maintain an increased barrier to poor-wetting liquids which only reach the outer layers of the hairs.

Measurements of Wetting of Hairs.

Various techniques were developed to measure the contact angle of different liquids on individual hairs of different insects. Insects were sprayed with a fine atomizer and were continuously observed under the microprojector apparatus described earlier. Some of the droplets impacted on hairs and scales, and their profile could be easily traced and the contact angles computed as before. Since the droplets were of very small size the effect of gravity was negligible. A large number of measurements of the advancing contact angle were made and means and standard errors calculated.

Sometimes the contact angle values determined in this manner cannot be relied upon for individual hairs because of the proximity of other hairs and scales. To overcome this difficulty, hairs of mosquitos and caterpillars were removed and allowed to fall on a glass slide freshly coated with nail varnish. After a few minutes the slide was carefully observed under the microscope and all the hairs which did not stick in upright positions were scraped off. The slide was passed through a spray mist and the contact angle determinations made as before, the image being enlarged to about 200 times the diameter of the original droplets.

An alternative technique was to immerse the insect in the test liquid (practicable only in the case of those liquids which partially wetted the cuticle) and pass bubbles of air through the liquid. By manipulation, small air bubbles were manoeuvred into contact with a body bristle, hair or scale. The projected outline of air bubbles resting in such a manner were traced and the contact angle computed from the data.

TABLE VI.

Contact angles in degrees of various liquids when applied to hairs of *Arctia caja*.

Liquid			Advancing angle	Receding angle
Odourless distillate	28.5 ± 1.0	25.3 ± 0.8
Non-aromatic	36.1 ± 0.7	31.0 ± 0.8
Low-aromatic	33.8 ± 1.0	29.4 ± 1.1
High-aromatic	32.6 ± 1.0	28.7 ± 0.9
Distilled water	97.3	90.2
P 31 (White oil)	42.7 ± 1.0	38.2 ± 1.1
Methyl phthalate...	52.8 ± 2.0	47.5 ± 1.5
Spray oil	29.7 ± 0.7	25.9 ± 0.7
Malariol	41.1 ± 1.01	35.1 ± 1.0
Olive oil	31.6 ± 0.78	28.0 ± 0.8
Shell A12	35.8 ± 1.0	31.2 ± 1.2
Shell K.B.30	39.0 ± 1.0	24.7 ± 0.9

In other experiments, hairs and scales were allowed to dip in a drop of test liquid resting on a horizontal surface and the contact angle measured directly from the meniscus of the liquid under the microprojector apparatus.

Lepidopterous larvae were most suitable for these tests because the hairs are comparatively large and easy to manipulate. The wetting of the hairs of caterpillars may not give reliable information of what happens on other insects, but these studies were designed to obtain some tentative information. The method used, was to spray detached hairs mounted on slides, as described above.

The average contact angle of twelve different liquids on hairs of tiger moth larvae (*Arctia caja*) are given in Table VI.

Further results are given in Table VII, from which it is clear that the contact angles on the hairs are significantly different from those on the dorsal integument of this insect; in fact they appear to resemble those on a plain beeswax surface.

TABLE VII.

Advancing contact angle in degree of various liquids when applied to beeswax surface and *Arctia caja* (both the integument and hairs).

Liquid	Contact angle in degrees		
	Beeswax surface	Dorsal side <i>A. caja</i>	Individual hairs <i>A. caja</i>
Distilled water	97	Approx. 180	97
Methyl phthalate	63	46	53
White oil P 31	39	Approx. 0	43
Spray oil	33	0	30
Odourless distillate	23	0	29

Both aqueous and oil droplets accumulate on hairs, although the two liquids do not wet the cuticle to the same degree. The droplets of aqueous solutions do not form a continuous surface film, and generally a high angle of contact is obtained, whereas oil droplets are formed by the coalescence of liquid after the preliminary formation of a surface film. This was demonstrated by the following experiment. A large number of droplets were allowed to impact on hair and spines of tiger moth larvae and, by means of a very fine needle, a minute quantity of oil soluble dye was added to one drop. The dye appeared in other drops within a few seconds, showing the presence of a thin surface film of oil.

3. INCORPORATION OF SURFACE ACTIVE AGENTS.

The present investigations were designed to obtain information on the effect of various surface active agents on the wetting and spreading of sprays when applied to the integument of insects and particularly on individual hairs.

Preliminary experiments were carried out on beeswax surface and insect integument and the contact angles measured by the microprojection apparatus described earlier (p. 123).

Beeswax.

Water soluble substances.

The contact angles for each concentration (0.05–10 per cent.) of different water soluble surface active agents are given in fig. 6. The data indicate :—

(i) Water soluble surface active agents greatly improve the wetting power of aqueous sprays and the contact angles decrease substantially.

(ii) With increasing concentration of surface active agents, the contact angles decrease until an optimum concentration is reached; beyond this concentration there is no further decrease, in fact there is a slight tendency to increase. The optimum concentrations of different surface active agents varied from 0.5 to 5.0 per cent.

(iii) It was found that varying the concentration of a surface active agent caused a relatively bigger change in contact angle than in surface tension. This confirms the observation of other workers.

(iv) Surface active agents such as CO9993, oil and water soluble, are of particular interest because according to Wigglesworth (1945) they disrupt the cuticular lipoids and perhaps improve permeability.

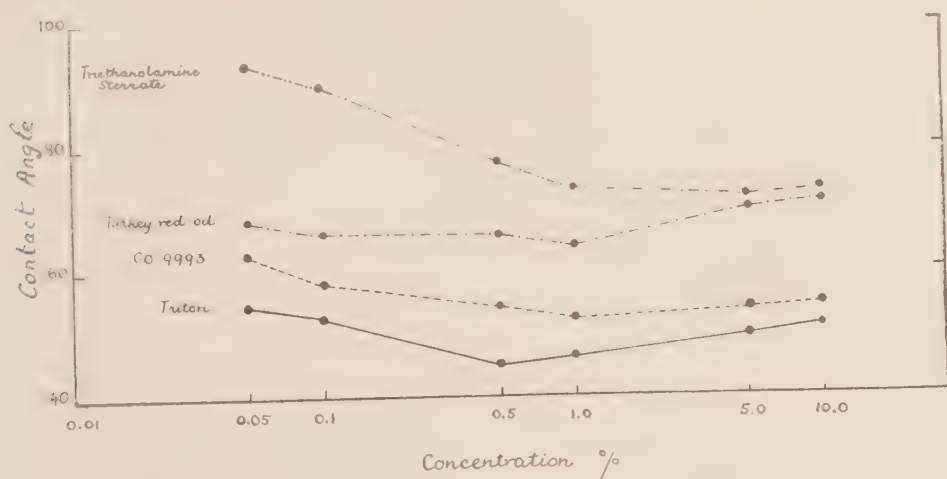


Fig. 6.—Water soluble surface active agents; contact angles of various concentrations on beeswax surface.

Oil soluble substances.

Only a rather small number of oil soluble surface active agents were obtainable at the time of this investigation. Contact angle measurements on standard beeswax surface for each concentration of those available are given in fig. 7.

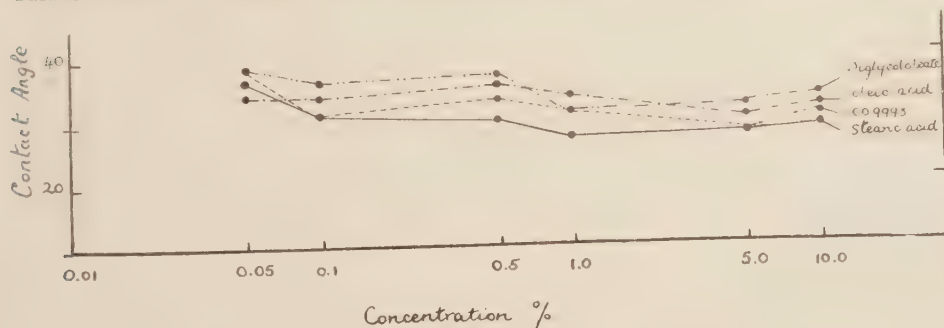


Fig. 7.—Oil soluble surface active agents; contact angles of various concentrations on beeswax surface.

As will be evident from fig. 7, the addition of surface active agents did not greatly affect the difference in contact angles of oils. There was a slight decrease at concentrations of about 5 per cent. (which would not be a commercial proposition). Oils have a low surface tension and it is difficult to reduce it. No practical advantage is therefore obtained by adding surface active agents to them.

Hairs.

Using the technique described above, the contact angles of 1 per cent. concentration of eight different surface active agents were measured on hairs of tiger moth larvae (*Arctia caja*), and the results are given in Table VIII. Although the aqueous sprays containing surface active agents readily wet the hairs, yet the droplets accumulate on them as described on p. 134.

TABLE VIII.

Contact angles in degrees of various water soluble surface active agents in distilled water when applied to hairs of *Arctia caja*. Concentration 1 per cent.

Surface Active Agent			Advancing Contact angle	Receding Contact angle
Sodium diglycol oleate	57.8 ± 2.7	49.76 ± 7.1
Emulsifier B 1956	39.3 ± 1.1	34.9 ± 1.0
Sodium oleate	46.4 ± 2.5	42.8 ± 2.3
CO9993	36.7 ± 1.2	31.8 ± 1.3
Triton NE	61.3 ± 2.1	57.4 ± 2.3
Teepol X	42.7 ± 1.4	37.5 ± 1.3
Triethanolamine stearate	55.1 ± 2.3	48.6 ± 2.3
Turkey red oil	48.3 ± 2.2	41.7 ± 2.0

CO9993	Cetyl ether of polyethylene glycol, $C_{16}H_{33}(OC_2H_4)_3OH$ (approx.).
Teepol X	Sodium secondary alkyl sulphates (Chain length $C_{10}-C_{18}$).
Triton NE	Polyethylene glycol monoiso-octyl tolyl ether.
B 1956	A phthalic glycerol alkyd resin.

A careful examination of these data will reveal no correlation between the contact angles produced by different surface active agents on beeswax and the hairs of *A. caja*. This contrasts with the strong correlation in contact angles of different liquids (Table VII).

TABLE IX.

Contact angles in degrees of various water soluble surface active agents when applied to beeswax surface, integument of *Tenebrio molitor* and hair of *Arctia caja*.

Surface Active Agents	Contact Angles		
	Concentration 1% Beeswax	Concentration 1-5% <i>T. molitor</i>	Concentration 1% <i>A. caja</i>
Distilled water	97	93	90-97
Triton NE	48	38.0	61.3
CO9993	50	38.36	36.7
Teepol X	65	55.48	42.7
Triethanolamine stearate	73	77.24	55.1
Turkey red oil	64	65.24	48.3
Sodium oleate	42	53.24	46.4
Sodium diglycol oleate	60	63.12	57.8
Emulsifier B 1956	51	42.84	39.3

As between *Tenebrio*/beeswax and *Tenebrio Arctia caja*, the contact angles of aqueous solutions of surface active agents show moderate correlation (Table IX). In order to examine the nature of insect cuticle lipoids by their wetting properties, the *Tenebrio* surface is perhaps more reliable than the other two investigated. (The beeswax surface is artificial and the results on *Arctia caja* may be influenced by the physical structure of the hairs.) Examining, then, the results with *Tenebrio*, it is found that the best wetters are the neutral un-ionised substances Triton NE and CO9993; less effective are basic ionised groups COONa (Teepol) and SO₃Na (Turkey Red Oil). Least effective was the neutral ionised Triethanolamine stearate. Cationic emulsifiers were, unfortunately, not available for investigation.

Summary.

The best method of assessing the wetting powers of liquids is to measure the contact angle formed with a particular solid surface. In order to study the wetting of insects by spray liquids, it was necessary to measure, as rapidly as possible, the contact angles of very small droplets on restricted surfaces (such as portions of the insects). The apparatus used projected a greatly enlarged image of the drop, the outline of which could be traced very quickly and used for subsequent calculations.

Under practical conditions, spray droplets impact on insects with some relative velocity either due to drift of the spray particles or to flight movements of the insect. Measurements were therefore made of the contact angles formed by droplets of water of known size falling on to insects (or to a beeswax surface) at a known speed. It was found that with rather large drops (5 mm. diameter) the contact angles formed were somewhat lower than the normal advancing contact angle. With small droplets (0.1-0.5 mm. diameter) there was no difference. Biological tests were made with 30 species of insects, differing widely in the nature of their integument and habitat. The resistance to wetting was found to vary greatly, not only from species to species but also on different parts of the body of a single insect. In general, most of the insects were readily wetted by oils and unwetted by water. Insects with hard cuticular lipoids, such as *Tenebrio*, were more hydrophobic than the Blattids with greasy cuticular waxes. A few species were both lipophilic and hydrophilic (larvae of *Mamestra*, *Polia*, *Musca*). Apart from the chemical nature of the cuticle, irregularities and the presence or absence of hairs were important. Increased roughness lowers the contact angles of liquids with good wetting powers, but has the opposite effect with liquids with poor wetting powers. Measurements were made of the contact angles formed on individual hairs of *Arctia caja* larvae by spray droplets. The contact angles formed on these hairs by plain liquids were strongly correlated with the angles formed on smooth *Tenebrio* cuticle or on an artificial beeswax surface; but there was only very rough correlation between the three sets of data when aqueous solutions of wetting agents were tested.

The test liquids included saturated and unsaturated hydrocarbons, mineral oils with varying aromatic contents, organic esters, alcohols and aqueous solutions. Among the hydrocarbons, members of the aliphatic series wet insect cuticle more readily than the aromatic group. The aromatic contents of the oils did not, however, affect their very high wetting powers.

The effects of adding surface active agents to aqueous sprays were investigated. Of the samples tested, the most effective wetting agents were those with neutral un-ionised molecules.

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References.

- ADAM, N. K. (1941). *The physics and chemistry of surfaces*. 3rd edn. Oxford Univ. Pr.
- BARTELL, F. E. (1941). Methods for measurement of contact angle.—*Chem. & Industry*, **160**, p. 475.
- BEAMENT, J. W. L. (1945). The cuticular lipoids of insects.—*J. exp. Biol.*, **21**, pp. 115–131.
- BEN-AMOTZ, Y. & HOSKINS, W. M. (1937). Factors concerned in the deposit of sprays. III. Effects of wetting and emulsifying powers of spreaders.—*J. econ. Ent.*, **30**, pp. 879–886.
- BROWN, G. T. & HOSKINS, W. M. (1939). Factors concerned in the deposit of sprays. V. The effects of pH upon the deposit of the oil and water phases of oil emulsions.—*J. econ. Ent.*, **32**, pp. 57–61.
- CASSIDY, A. B. D. & BAXTER, S. (1945). Large contact angles of plant and animal surfaces.—*Nature, Lond.*, **155**, pp. 21–22.
- DAVID, W. A. L. (1946*a*). The quantity and distribution of spray collected by insects flying through insecticidal mists. *Ann. appl. Biol.*, **33**, pp. 133–141.
- DAVID, W. A. L. (1946*b*). Factors influencing the interaction of insecticidal mists and flying insects. Part II. The production and behaviour of kerosene base insecticidal spray mists and their relation to flying insects.—*Bull. ent. Res.*, **37**, pp. 1–28.
- DUNN, P. L. (1926). *Surface equilibria of biological and organic colloids*. New York, Chem. Catalog Co.
- EBELING, W. (1939). The rôle of surface tension and contact angle in the performance of spray liquids.—*Hilgardia*, **12**, pp. 665–698.
- ENGLISH, L. L. (1928). Some properties of oil emulsions influencing insecticidal efficiency.—*Bull. Ill. nat. Hist. Surv.*, **17**, pp. 233–259.
- EVANS, A. C. & MARTIN, H. (1935). The incorporation of direct with protective insecticides and fungicides. I. The laboratory evaluation of water-soluble wetting agents as constituents of combined washes.—*J. Pomol. hort. Sci.*, **13**, pp. 261–292.
- FOGG, G. E. (1947). Quantitative studies on the wetting of leaves by water.—*Proc. roy. Soc., (B)* **134**, pp. 503–522.
- HAMILTON, C. C. (1930). The relation of the surface tension of some spray materials to wetting and the quantity of lead arsenate deposited.—*J. econ. Ent.*, **23**, pp. 238–251.
- HENSILL, G. S. & HOSKINS, W. M. (1935). Factors concerned in the deposit of sprays. I. The effect of different concentrations of wetting agents.—*J. econ. Ent.*, **28**, pp. 942–950.

- HOSKINS, W. M. (1940). Recent contributions of insect physiology to insect toxicology and control.—*Hilgardia*, **13**, pp. 307-386.
- HOSKINS, W. M. & WAMPLER, E. L. (1936). Factors concerned in the deposit of sprays. II. Effect of electrostatic charge upon the deposit of lead arsenate.—*J. econ. Ent.*, **29**, pp. 134-143.
- LANE, W. R. (1937). A microburette for producing small liquid drops of known size.—*J. Sci. Instrum.*, **24**, pp. 98-101.
- MACK, G. L. (1936). The determination of contact angles from measurements of dimension of small bubbles and drops.—*J. phys. Chem.*, **40**, pp. 159-167, 169-175.
- O'KANE, W. C., WESTGATE, W. A., GLOVER, L. C. & LOWRY, P. R. (1930). Studies of contact insecticides. I. Surface tension, surface activity and wetting ability as factors in the performance of contact insecticides.—*Tech. Bull. N.H. agric. exp. Sta.*, no. 39, 42 pp.
- O'KANE, W. C., WESTGATE, W. A. & GLOVER, L. C. (1932). Studies of contact insecticides. V. The performance of certain contact agents on various insects.—*Tech. Bull. N.H. Agric. exp. Sta.*, no. 51, 20 pp.
- SCHMIDT, W. (1909). Eine unmittelbare Bestimmung der Fallgeschwindigkeit von Regentropfen.—*Met. Z.*, **26**, p. 183.
- STELLWAAG, F. (1924). Die Benetzungsfähigkeit flüssiger Pflanzenschutzmittel und ihre direkte Messbarkeit nach einem neuen Verfahren.—*Z. angew. Ent.*, **10**, pp. 163-176.
- THORPE, W. H. & CRISP, D. J. (1947). Studies on plastron respiration. I. The biology of *Aphelocheirus* (Hemiptera, Aphelocheiridae (Naucoridae) and the mechanism of plastron retention.—*J. exp. Biol.*, **24**, pp. 227-269.
- WAMPLER, E. L. & HOSKINS, W. M. (1939). Factors concerned in the deposit of sprays. VI. The role of electrical charges produced during spraying.—*J. econ. Ent.*, **32**, pp. 61-69.
- WENZEL, R. N. (1936). Resistance of solid surfaces to wetting by water.—*Industr. Engng Chem.*, **28**, pp. 988-994.
- WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects.—*J. exp. Biol.*, **21**, pp. 97-114.
- WIGGLESWORTH, V. B. (1947). The epicuticle in an insect, *Rhodnius prolixus* (Hemiptera).—*Proc. roy. Soc., (B)* **134**, pp. 163-181.
- WILCOXON, F. & HARTZELL, A. (1931). Some factors affecting the efficiency of contact insecticides. I. Surface forces as related to wetting and tracheal penetration.—*Contr. Boyce Thompson Inst.*, **3**, pp. 1-12.

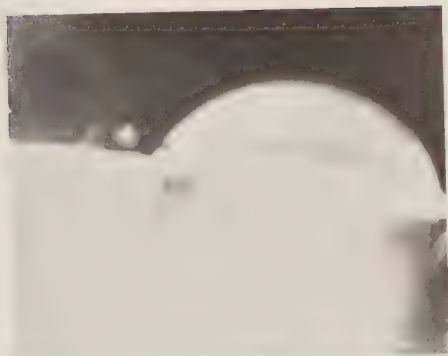


FIG. 1. Projected outline of drops of distilled water on *Tenebrio molitor*.

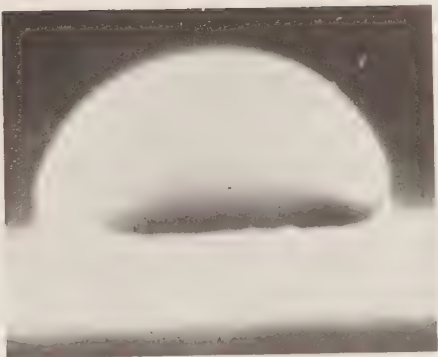


FIG. 2. Projected outline of drops of distilled water on beeswax surface.

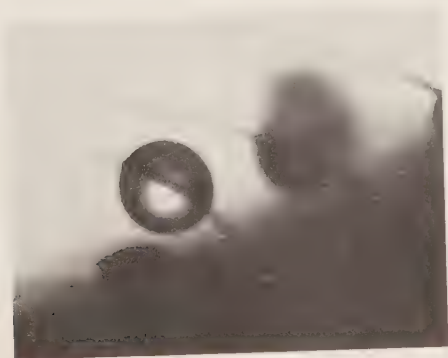


FIG. 3. Projected outline of oil droplets on hairs of *Culex pipiens*.

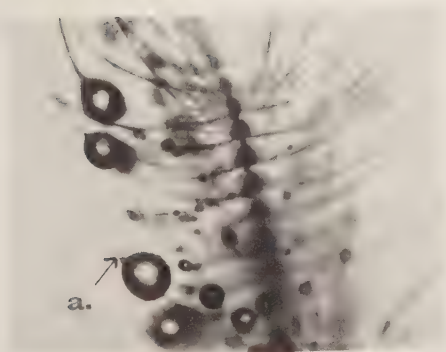


FIG. 4. Oil droplets clinging to antennal hairs of *Culex pipiens*.
(a) Six hairs pulled together due to surface tension.



FIG. 5. Oil droplets clinging to abdominal hairs of *Culex pipiens*.

THE ACTION OF ROTENONE AND TETRAETHYL PYROPHOSPHATE ON THE ISOLATED HEART OF THE COCKROACH.*

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The functional activity of the heart of vertebrates has been thoroughly studied for many years and much has become known regarding the action of the automatic centres and the extracardiac nervous regulation. On the contrary, not so much is known about the physiology of the heart of invertebrates; for instance, relatively few research workers have investigated the action of the insect heart. As regards the automatism of the insect heart, Alexandrowicz (1926) supposed that it originated in the paired lateral cardiac nerve. In the case of *Melanoplus* and *Periplaneta*, Walling (1908) and Steiner (1932) found ganglion cells in the lateral cardiac nerves and the impulses generated by these cells would act directly upon the cardiac muscles.

As is well known, the insect heart, unlike the heart of the vertebrates, functions by means of two antagonistic muscle systems, the sphincters in the wall of the heart and the alary muscles. Some research workers are of the opinion that the alary muscles are not induced to rhythmic contraction by nerve impulses, but by means of the mechanical extension due to the heart contraction (*cf.* Dubuisson, 1929). There are even authors who hold that the alary muscles are not contractile, but merely elastic. In fact, in some cases (*e.g.* *Periplaneta*) electric stimulation of the isolated alary muscles does not cause any contraction (Yeager, 1939; de Wilde, 1947). However, in other cases contraction of the lateral muscles, as a result of electric stimulation, has been observed (*cf.* de Wilde, *Cossus* larvae). As the alary muscles are cross striated, also in *Periplaneta*, and are innervated by the lateral cardiac nerves (Alexandrowicz, 1926), it seems improbable that they would have no contractile properties. Hamilton (1939) and de Wilde (1947) did directly observe the rhythmic contraction of the alary muscles under physiological conditions.

While the data are not yet complete, the impression is very strong that the insect heart has a neurogenic automatism, in which the ganglion cells of the lateral cardiac nerves function as a source of the automatism. In the case of Crustacea the neurogenic origin of the heart automatism is unquestionable (*cf.* Welsh & Schallek, 1946). There is, however, a possibility, that the neurogenic automatism of the Crustacean heart is of a secondary nature and covers a primary myogenic automatism (*e.g.*, Duwez, 1938).

As regards the extracardiac nervous regulation of the insect heart, Carlson (1905) has established that the cerebral and the thoracic ganglia may exert an accelerating as well as an inhibitory influence upon the heart frequency. Sasse (1911) observed that stimulation of the other ganglia does not affect the heart beat. Lasch (1913) found in some cases no influence but in others an inhibitory influence after stimulation of the cerebral ganglion. Steiner (1932) could find neither inhibitory nor accelerating fibres. It is not yet quite clear, but, if the condition is the same as in the Crustacea (*cf.* Welsh & Schallek), one is justified in suspecting, that insects, too, have extracardiac accelerating and inhibitory nerves.

Our imperfect knowledge of the physiology of the insect heart undoubtedly is partly owing to the fact that the heart is very fragile and can only be examined in an

*This investigation was carried out under the auspices of the National Council for Agricultural Research (Toegepast Natuurwetenschappelijk Onderzoek), Holland.

isolated state with the utmost difficulty. Complete isolation is not feasible, as the alary muscles cannot function without connections to the body wall. Some authors have succeeded in keeping the heart beating after the removal of all other organs, so that only the dorsal wall of the body remains with the heart attached thereto. In this way Levy (1928) examined the influence of ions upon the heart of fly larvae, but the frequency of the heart beat did not remain constant in his experiments and consequently quantitative conclusions were not possible. In a similar way Yeager and Hager (1934), Yeager and Gahan (1937) and Yeager (1938) studied the surviving heart of *Periplaneta americana* (L.) and *Prodenia eridania* (Cram.) in a physiological solution at a constant temperature. Yeager and Gahan, Uramoto (1932), Duwez (1938), Hamilton (1939), Crescitelli and Jahn (1938) and de Wilde (1947) also succeeded in mechanically recording the activity of the insect heart.

Mechanical recording is doubtless an excellent means of studying some problems relating to the function of the heart but it is not a method that can always be recommended. For instance, if the influence of drugs, ions and toxic substances over prolonged periods of time has to be studied, it is necessary to keep the heart beating in a normal rhythm for hours and, as mechanical registration hinders the fragile heart muscles, this cannot always be achieved. In such cases direct observation by Yeager's method is more suitable.

Yeager's experimental conditions are not fully satisfactory, for while the heart frequency of *P. americana* in the intact animal is 80-110 per minute at 30°C. (Brücke, 1925), Yeager states, that the frequency in his preparations soon falls to 40-50 per minute. However, by Yeager's method it has been possible to obtain experimental conditions, in which the heart maintains a normal rhythm over prolonged periods of time.

Methods.

Adults of *P. americana* were used. The insect was immersed in water until it was inactivated, the head and legs were then cut off and it was pinned to a wax plate, ventral side uppermost. The wax plate, in turn, was pinned down in a preparation basin containing a physiological solution.

This physiological solution (according to Yeager) is a modified Ringer's solution containing: NaCl 9.82 gm., KCl 0.77 gm., CaCl_2 0.50 gm., NaHCO_3 0.18 gm., NaH_2PO_4 0.01 gm., dextrose 1 gm., aqua dest. ad 1000 cc. The pH of the solution should be between 7.0 and 7.5 and this can be corrected, if necessary, by means of NaOH or HCl, for the regularity of the heart beat is highly dependent upon the pH.

The insect was cut open along the median line, and the abdominal skin, after the adherent tissue had been loosened, was folded to either side and cut off. The intestine should not be damaged, as the intestinal fluid might be injurious to the heart beat; it was squeezed at both ends by means of tweezers, cut close to the anus and removed. Fat tissue and genitalia were then carefully removed until the heart was clearly visible. The wax plate with the object was then introduced vertically into an experimental tube, which was placed in a water bath (fig. 1) kept at a constant temperature of 30°C.

A constant stream of Ringer's solution was allowed to drip into a funnel that reaches down to the bottom of the experimental container and an outlet kept the solution in the experimental tube at a constant level. The solution was aerated, thus providing oxygen and agitation. The heart beat could easily be studied by means of a binocular microscope and the frequency determined with a stop watch. Changes in frequency and amplitude, as well as systolic and diastolic types of contraction, were quite clearly perceptible.

The heart generally stops or beats irregularly at first (*cf.* fig. 2A) and some 30 minutes elapse before it overcomes the post-operative shock. At the end of that period the heart beat becomes regular in about 70 per cent. of the preparations and maintains a normal frequency of 80-110 per minute for hours (sometimes for 24 hours, *cf.* fig. 2A). Only hearts beating in a strong and normal way were used for the experiments.

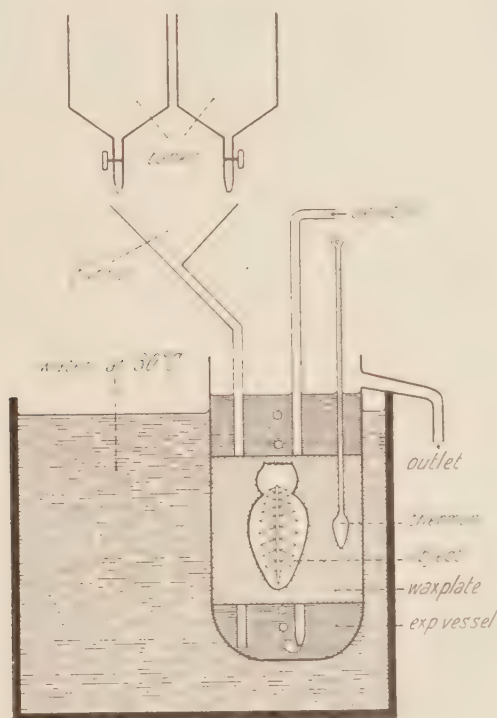


Fig. 1.—Apparatus for studying the isolated insect heart.

A constant supply of Ringer's solution is important; when this becomes slower than about two drops per second, a decrease of the heart frequency soon sets in. Fig. 2B shows that a cessation of the supply may even cause the heart to stop but with the renewal of the supply the heart regains its original rhythm. The aeration is also important for normal functioning.

The insect heart, prepared in this way, is influenced only by the automatic centres in the lateral cardiac nerves. Extracardiac nervous regulation by other ganglia is impossible, as these have been removed during the preparation. The preparation therefore lends itself to the study of the automatism and the effect upon it of temperature, ions, drugs, etc. Some technical details of the administration of such substances, which may be dissolved in Ringer's solution, should be mentioned.

It is not desirable to transfer the heart from the normal Ringer's solution to another container filled with the experimental solution, for the effect of this mechanical disturbance is in itself sufficient to cause irregularities in the heart rhythm, which may last for minutes. The correct procedure is to wait until the heart has overcome the post-operative shock and beats steadily, and then to stop

the supply of normal Ringer's solution and from another container (cf. fig. 1) to add the experimental solution, preheated to 30°C. The experimental solution flows in rapidly until the normal solution in the experimental container has been quite replaced, when the supply is reduced to drips.

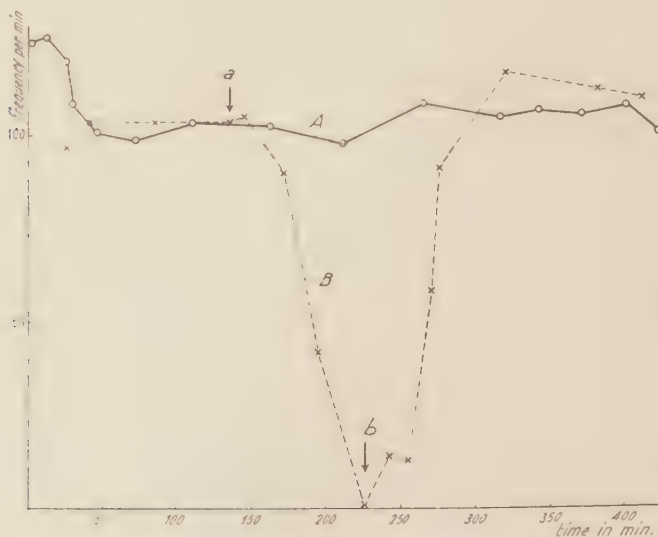


Fig. 2 (A).—Frequency of the surviving heart of *Periplaneta* under favourable experimental conditions. About 30 mins. after the introduction into the experimental tube, the frequency becomes approximately 100 per minute and then remains constant for hours at a time.

(B) (dotted line).—At the arrow *a* Ringer perfusion is stopped, the frequency decreases and the heart stops after some time. At the arrow *b* the Ringer supply is resumed, the heart begins to beat again and gradually regains its normal frequency.

The Action of Rotenone and Derris Extracts.

The well known insecticide rotenone seems in vertebrates to attack in the first place the respiration centre of the *medulla oblongata*, according to von Hasselt (1910, 1911), Haag (1921) and Ambrose and Haag (1936, 1937, 1938); the automatism of the heart is affected only at a much higher concentration. It would seem, therefore, that nervous centres are more sensitive than the myogenic centres of the heart. In invertebrates, nothing is known of the primary point of attack of rotenone and it was, therefore, desirable to investigate to what extent the nervous centres of automatic functions in insects are affected. As the heart automatism in insects is probably of neurogenic origin, there is a possibility that the insect heart, like the respiration centre in vertebrates, is very sensitive to rotenone. In order to study this, experiments were carried out using the desired quantity of pure crystalline rotenone dissolved in absolute alcohol or acetone. One cc. of this solution was added to 500 cc. of Ringer's solution and supplied to the isolated heart in the manner described above. Control tests established that the low alcohol or acetone concentration in the Ringer's solution has no influence upon the action of the heart. Rotenone, however, brings about marked phenomena; the contractions become diastolic and, almost simultaneously, the frequency falls, leading to stopping of the heart in diastole (cf. fig. 3). Even a rotenone concentration as low as 0.000005 per cent. results in a decrease of the frequency and cessation of the heart. The heart automatism,

therefore, appears to be very sensitive indeed to rotenone and the surmise that the ganglion cells in the lateral cardiac nerves are inhibited in their function by rotenone is obvious. As there is a decrease of the frequency as well as of the amplitude of the heart beat, it is plausible that rotenone decreases the frequency of the impulse volleys from the ganglion cells and also the number of impulses per volley so that not all muscle fibres proceed to contract. Crescitelli and Jahn (1938), Wiersma and Novitski (1942) and Armstrong & others (1939) found indications of a tetanic character for the heart beat in Arthropods; a suppression of the number of impulses per volley resulting in a weakened contraction, therefore, is quite possible. Further research will have to determine whether this is the case and it is hoped to return to the subject in a future publication.

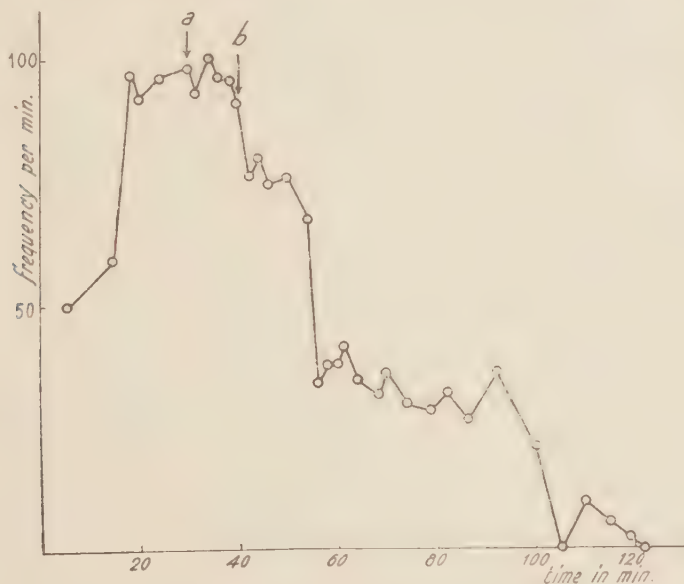


Fig. 3.—At the arrow *a* normal Ringer's solution is replaced by 0.000002% of rotenone in Ringer.

At the arrow *b* the contractions become diastolic, very soon thereafter the frequency falls and finally the heart stops in diastole.

The appearance of the diastolic contractions is more useful as an indicator of the action of rotenone upon the heart than the decrease of the frequency. As has been pointed out, in the normal heart the frequency may vary between certain limits. The first part of the decrease of frequency may fall within the normal limits and therefore is not always recognized as the beginning of the rotenone action. On the contrary, the appearance of diastolic contractions is a distinct indication of the beginning of the rotenone action (*cf.* fig. 3*b*) as the normal heart shows no diastolic contractions.

In the course of the investigations it was found that the period of time between the administration of the rotenone solution and the appearance of the diastolic contractions depends upon the concentration used. Diastolic phenomena and decrease of frequency appear later, as the rotenone concentration is lower. In Table I the results of a number of tests using different rotenone concentrations are given; the dependence of the latent period upon the concentration used is clearly visible herein.

When the average value of the latent period is plotted against the concentration, the hyperbolic curve shown in fig. 4A results. That this curve is indeed a hyperbole, at any rate in the middle part, is evidenced by the fact that the product of concentration and latent period is constant.

In Table I this product has been given for each of the concentrations used. It will be seen that at very high and at very low concentrations the constant begins to deviate, as the experimental error becomes bigger in those ranges. But the middle part of the curve, between 0.000002 and 0.000008 per cent. of rotenone, meets the requirements of a hyperbole sufficiently well.

TABLE I.

Latent period in minutes upon administration of different concentrations of rotenone.

Conc. Rotenone	0.000001%	0.000002%	0.000003%	0.000004%	0.000005%	0.000008%	0.000010%
	25.5	8.5	5.5	4	4.5	2.5	2
	19	9	5	5	4	1.5	4
	17	9	6	4.5	3	3	2
	17	9	7	5.5	3	2.5	2
	18	9.5	6	6	3	2	2.5
	22.5	10.5	6	5	4	3	2.5
	20	8.5	7	4.5	3	1.5	3
	21.5	7.5	5.5	4	4	2.5	4
		9.5	7		3.5	3	2
Average latent period	20.1	9	6.1	4.8	3.6	2.2	2.7
Constant (c.t.)	0.0000201	0.0000180	0.0000183	0.0000192	0.0000180	0.0000176	0.0000270

The fact that in every case a constant quantity (c.t.) of rotenone must be added to the heart before the influence becomes visible, indicates that rotenone is accumulated in the tissues (probably in the ganglion cells) and starts its action as soon as it has attained a critical concentration therein. This opinion is supported by the irreversibility of the poisoning process; once rotenone has evoked diastolic contractions, a return to normal Ringer's solution will not enable the heart to recover. Therefore, rotenone is strongly retained in the tissues and even the administration of very weak solutions will finally, by accumulation, result in a toxic concentration.

From the foregoing it will be seen that the latent period is a measure of the rotenone concentrations used. The question arose, therefore, if the estimation of derris preparations with an unknown rotenone content would be possible in this way. The content of the active substance in derris roots varies widely and each new batch should therefore be tested, an operation that is usually carried out chemically. In addition to rotenone, the derris root contains other chemically closely related insecticides, amongst others, sumatrole, toxicarole and degueline, the chemical determination of which is very incomplete. The method of the surviving insect heart, however, gave an opportunity to determine the total activity of derris powder in a relatively simple way. Derris powder was extracted in acetone, the extract diluted with absolute alcohol and 1 cc. of the solution added to 500 cc. of Ringer's solution. It was found that derris extract produced the same symptoms as pure rotenone (diastolic contractions, decreased frequency) and when use was being made of different concentrations, again and again the product of latent period and concentration was found to be constant over a great range of concentrations so that a hyperbolic curve was obtained (*cf.* fig. 4B).

In order to compare an unknown derris preparation with pure rotenone, tests should be carried out with the unknown preparation at four or five different concentrations from the middle of the hyperbole. With each of these concentrations the latent period should be determined at least six times and the average of these values taken; for each of the concentrations the constant c.t. should be calculated, the average taken and compared with the constant for pure rotenone. Assuming the activity of pure rotenone to be 100 per cent., the activity of the unknown derris preparation may be calculated from the equation
$$\frac{\text{c.t. (rotenone)}}{\text{c.t. (derris)}} \cdot 100 \text{ per cent.} = \text{activity in per cent. of rotenone.}$$
 Results obtained in this way were confirmed in many of the tests.

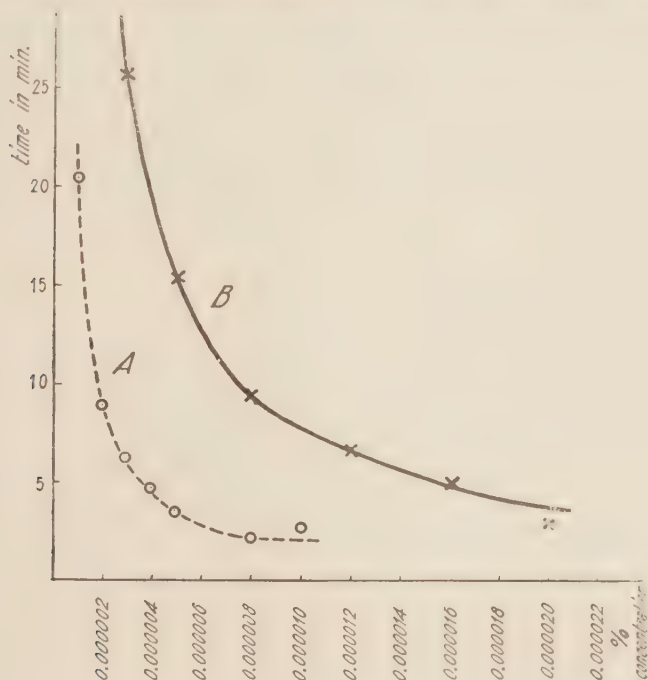


Fig. 4.—Relation between latent period and concentration. The values obtained are disposed on a hyperbole (c.t. = k). (A) Rotenone; (B) Derris powder extract.

TABLE II.

Preparation	Chemical analysis		Activity upon the heart in % of rotenone	Activity in % of rotenone upon injection
	rotenone %	resin remainder %		
A	3.5	15.4	5.0	4.5
B	5.9	10.0	7.5	7.7
C	6.9	16.4	8.0	7.3
D	7.8	16.5	14.0	14.1

Some tests carried out with chemically well-known derris preparations are listed in Table II. It will be seen that the chemical analysis of the rotenone content does

not correspond to the activity upon the heart. The latter is always higher than might be expected in view of the rotenone content, thus indicating that there are substances in the resin remainder also affecting the activity of the heart.

Whether the activity thus determined is identical with the toxicity of the preparations, that is whether the value obtained is really a measure of the total content of insecticide components, remains to be solved. It may be that the other insecticide components act differently from rotenone upon the heart. It has not been possible to obtain the other insecticide components in pure form and consequently the individual activity of these substances has not been ascertained. It is improbable, however, that their activity is different because derris extract produces the very same phenomena in the heart as pure rotenone but it was, nevertheless, desirable to compare the method of the surviving heart with another quantitative method. This is possible by means of the injection method. Injections of different derris concentrations into several hundred cockroaches permitted the determination of the mortality curve of an unknown preparation with precision and the calculation of the LD50. The LD50 of pure rotenone (*cf.* Dresden & Krijgsman 1948) could then be compared with the LD50 of the unknown preparation and the rotenone activity of the latter could be calculated. From Table II it will be seen that the results obtained by the injection tests and the heart method correspond closely. The heart method may, therefore, be considered reliable.

The Action of Tetraethyl Pyrophosphate.

The high toxicity of alkylpolyphosphates according to Chadwick & Hill (1947), Dubois & Mangun (1947) and Burger & others (1947), is entirely, or for the most part, due to their property of exerting a destroying action upon the tissue cholinesterases. The excess of acetylcholine, thus produced, would cause marked disturbances in the function of the muscle nerve system. The action of these phosphates must be more or less comparable, therefore, to that of other anticholinesterases such as eserine and the symptoms are similar to those evoked by the administration of acetylcholine. In vertebrates, among the outstanding symptoms of intoxication by anticholinesterases are inhibition of the heart beat, excitation of the respiratory centre and increased activity of the *medulla spinalis* in general. In Crustacea, aserine and acetylcholine have an accelerating influence upon the heart frequency, and acetylcholine would, therefore, have a directly activating influence upon the nerve cells of the cardiac ganglion (Welsh & Schallek, 1946) in the same way that it acts upon the respiratory centre in vertebrates. In insects, Roeder & Roeder (1939) have shown that eserine as well as acetylcholine considerably increases the activity of the abdominal nerve chord of *Periplaneta americana*; Roeder (1948) saw a facilitation of synapses, followed by block, in the nerve chord of *Periplaneta*, caused by an anticholinesterase (di-isofluorophosphate). Richards & Cutkomp (1945), like Chadwick & Hill (1947), found cholinesterases in the nervous tissue of insects, which makes the activating effect of anticholinesterases understandable. Finally, Hamilton (1939) observed that the heart of *Melanoplus* is accelerated by acetylcholine. In view of the foregoing it may, therefore, be expected that the anticholinesterase tetraethyl pyrophosphate (TEPP) would also cause an acceleration of the heart frequency in *Periplaneta*.

Tests carried out with practically pure TEPP* dissolved in Ringer's solution gave the following results :—

Concentrations of 0.02 per cent. and higher immediately caused systolic contractions, decrease of the frequency and soon cessation in systole. The reason for this is probably the acid character of the solution and is very probably not specific as the heart is very sensitive to an acid medium and weak hydrochloric acid concentrations cause exactly the same phenomena. What is remarkable is

*Kindly placed at our disposal by Prof. J. A. A. Ketelaar, Amsterdam.

that there follows a range of concentrations in the neighbourhood of 0.008 per cent., which is inactive. Even after long perfusion these TEPP-concentrations have no influence on the heart. Presumably the inhibitory action and the accelerating action of the weak concentrations discussed below just compensate each other.

Weak concentrations (from 0.004 up to 0.000008 per cent.) give a sharp rise of the frequency (fig. 5), usually accompanied by a slight increase in amplitude. The rise in frequency sets in immediately after administration and after a short time usually regains a constant higher level. Lower concentrations than 0.000008 per cent. have no longer any effect.

The fact that there is no latent period, not even in the weakest active concentrations, is characteristic. The addition of TEPP either produces an immediate effect, or, when the concentration is too weak, none at all, not even after prolonged perfusion. This indicates, that, unlike rotenone, there is no accumulation in the nerve tissue. Owing to the absence of the latent period it is not possible to estimate the TEPP-content in technical preparations in the same way as has been described for rotenone. Perhaps it would be possible to determine the TEPP-content in commercial preparations by the determination of the threshold value, but these tests take up much time. Other methods such as chemical analysis and the contact method (Krijgsman & Berger, 1949) are quicker.

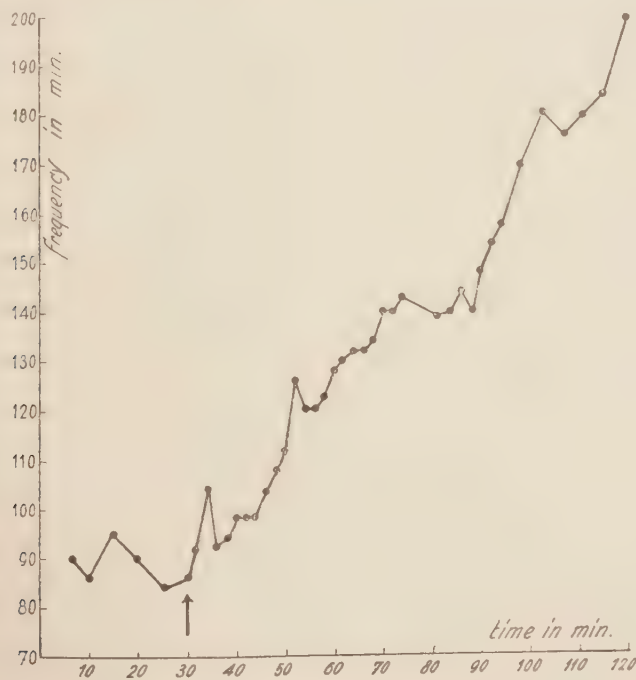


Fig. 5.—Rise of frequency of the heart under the influence of TEPP, administrated at the arrow (0.00004 per cent.).

It is concluded, in agreement with other research workers, that TEPP develops a vigorous anticholinesterase activity. In the first place this is apparent from the fact that acetylcholine in physiological concentrations (threshold 0.000005 per cent.) will raise the heart frequency in exactly the same way as TEPP. Moreover

the heart is sensitised to acetylcholine by TEPP-concentrations too weak to produce an effect. As regards these experiments and tests with other drugs, it is hoped that more detailed information will be given in a future publication.

The anticholinesterase action of TEPP is certainly the principal cause of its insecticidal activity. At the same time other possibilities must not be precluded; Mommaerts (1948), for instance, found that organic phosphates exerted a strong influence upon the viscosity of myosin, so it is not excluded that alkylpolyphosphates, in addition to their anticholinesterase action, also have a direct influence on muscle contractility.

Summary.

A method based upon Yeager's technique is described permitting the study of the surviving heart of *Periplaneta americana* under varying conditions for several hours.

Rotenone causes diastolic contractions, decrease of frequency and cessation in diastole, threshold = 0.0000005 per cent. Presumably it produces an inhibitory action on the neurogenic automatic cardiac centre.

The latent period of the rotenone action is dependent upon the concentration. Using this principle a method is developed for quantitative estimation of the activity of derris preparations.

Tetraethyl pyrophosphate has a strongly accelerating action on the frequency of the heart and increases the amplitude; in this case there is no latent period. Threshold 0.000008 per cent. Presumably TEPP activates the neurogenic heart automatism by its vigorous anticholinesterase activity.

References.

- ALEXANDROWICZ, J. S. (1926). J. comp. Neurol., **41**, p. 291.
 AMBROSE, A. M. & HAAG, H. B. (1936). Industr. Engng Chem., **28**, p. 315.
 AMBROSE, A. M. & HAAG, H. B. (1937). *Ibid.*, **29**, p. 429.
 AMBROSE, A. M. & HAAG, H. B. (1938). *Ibid.*, **30**, p. 592.
 ARMSTRONG, F., MAXFIELD, M., PROSSER, C. L. & SCHOEFFLE, S. (1939). Biol. Bull., **77**, p. 327.
 BETHE, A. (1926). In Handb. norm. path. Physiol., **7**, p. 1.
 VON BRÜCKE, E. Th. (1925). In Winterstein Handb. vergl. Physiol., **1** (1) p. 898.
 BURGER, A. S. V., KEELE, C. A., CHENNELLS, M., DEL CASTELLO, J., FLOYD, W. F., SLOME, D. & WRIGHT, S. (1947). Nature, Lond., **160**, p. 760.
 CARLSON, A. J. (1905). Amer. J. Physiol., **15**, p. 127.
 CHADWICK, L. E. & HILL, D. L. (1947). J. Neurophysiol., **10**, p. 235.
 CRESCITELLI, F. & JAHN, Th. J. (1938). J. cell. comp. Physiol., **11**, p. 359.
 DRESDEN, D. & KRIJGSMAN, B. J. (1948). Bull. ent. Res., **38**, p. 575.
 DUBOIS, K. P. & MANGUN, G. H. (1947). Proc. Soc. exp. Biol. Med., **64**, p. 137.
 DUBUISSON, M. (1929). Arch. Biol., **39**, p. 247.
 DUWEZ, Y. (1938). Arch. int. Physiol., **46**, p. 389.
 FRIES, E. F. S. (1926). J. gen. Physiol., **10**, p. 227.
 HAAG, H. B. (1921). J. Pharmacol., **43**, p. 193.
 HAMILTON, H. L. (1939). J. cell. comp. Physiol., **13**, p. 91.
 VON HASSELT, E. H. (1910). Versl. gewone Vergad. Akad. Amst., **19**, p. 704.

- VON HASSELT, E. H. (1911). Arch. int. Pharmacodyn., **21**, p. 243.
- KIRSCHNER, R. (1932). Z. angew. Ent., **19**, p. 544.
- KREY, J. (1937). Zool. Jb. (Physiol.), **58**, p. 201.
- KRIJGSMAN, B. J. & BERGER, N. E. (1949). Bull. ent. Res., **40**, p. 355.
- LASCH, W. (1913). Z. allg. Physiol., **14**, p. 312.
- LEVY, R. (1928). C. R. Soc. Biol., **99**, p. 1482.
- MOMMAERTS, W. H. F. M. (1948). J. gen. Physiol., **31**, p. 361.
- RICHARDS, A. G. & CUTKOMP, L. H. (1945). J. cell. comp. Physiol., **26**, p. 57.
- ROEDER, K. D. (1948). J. cell. comp. Physiol., **31**, p. 327.
- ROEDER, K. D. & ROEDER, S. (1939). J. cell. comp. Physiol., **14**, p. 1.
- SASSE, E. (1911). Z. allg. Physiol., **13**, p. 69.
- SHAFFER, G. D. (1911). Tech. Bull. Mich. agric. Exp. Sta. no. 11, 65 pp.
- STEINER, G. (1932). Z. vergl. Physiol., **16**, p. 290.
- URAMOTO, S. (1932). Bull. seric. Exp. Sta. Japan, **8**, p. 121.
- WALLING, L. V. (1908). Kans. Univ. Sci. Bull., **4**, p. 359.
- WELSH, J. H. & SCHALLEK, W. (1946). Physiol. Rev., **26**, p. 447.
- WIERSMA, C. A. G. & NOVITSKI, E. (1942). J. exp. Biol., **19**, p. 255.
- DE WILDE, J. (1947). Arch. néerl. Physiol., **28**, p. 530.
- YEAGER, J. F. (1938). J. agric. Res., **56**, p. 267.
- YEAGER, J. F. (1939). *Ibid.*, **59**, p. 121.
- YEAGER, J. F. & GAHAN, J. B. (1937). J. agric. Res., **55**, p. 1.
- YEAGER, J. F. & HAGER, A. (1934). Iowa St. Coll. J. Sci., **8**, p. 391.
- YEAGER, J. F., HAGER, A. & STRALEY, J. H. (1935). Ann. ent. Soc. Amer., **28**, p. 256.
- ZAWARSIN, A. (1911). Z. wiss. Zool., **97**, p. 481.
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THE RESTING HABITS OF *GLOSSINA MEDICORUM*, *G. FUSCA* AND *G. LONGIPALPIS*.

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(Plates IV and V.)

The purpose of this note is to illustrate the importance of searching for resting tsetse when making a fly survey. Highly experienced entomologists and fly boys, who are accustomed to making surveys for *G. palpalis* (R.-D.), *G. tachinoides* Westw. and *G. morsitans* Westw., get into the habit of expecting tsetse to be attracted to themselves or to screens or to bait animals, and may easily fail to detect the less common species unless they appreciate that these should be searched for in their resting haunts.

in areas in
which *Glossina*
medicorum, *G.*
fusca, and *G.*
longipalpis
may occur

The observations recorded below were made over four days which were spent in the Olokemeji Forest Reserve in late October 1949, when the rains were ending; the reserve has an area of about 25 square miles. The vegetation mainly consists of high, mixed deciduous forest but in places the forest has been underplanted with introduced species and in other parts has been replaced by plantations of teak (*Tectona grandis*). When driving down the road which passes through the reserve, one would imagine that one was well within the forest belt, but actually the woodland savannah is never very far distant. Olokemeji is situated on the borders of the Derived Savannah and Dry Forest belts. (For a description of these vegetation zones the reader should consult Keay, 1949.)

For many years it has been known that *G. palpalis*, *G. longipalpis* Wied. and *G. fusca* (Wlk.) occurred within the reserve; in February 1948 one specimen of *G. medicorum* Aust. was taken.

The observations made on this recent survey will be recorded separately for each species.

***Glossina medicorum* Austen.**

The following historical notes serve to illustrate how easily *G. medicorum* may be overlooked, even though abundant. A highly skilled entomologist had lived for some 24 years within 24 miles of the Olokemeji reserve, knew the area well, and had collected tsetse there, but neither he nor his assistants ever took this species. In February 1948, a specimen was taken quite fortuitously by one of our staff. Subsequently, a highly skilled tsetse entomologist who was particularly keen to take this species visited the reserve on two occasions, but although he took 17 *G. fusca* he failed to take *G. medicorum*. Shortly afterwards he arranged for five fly catchers to spend a week in the reserve. They took numerous *G. fusca* but only two *G. medicorum*; incidentally, they spent two whole days without success in the same area where, using the technique to be described below, this species was subsequently found to be numerous. On 24th October 1949, the writers and six other catchers using

this black and hessian screens failed to take this species, as did a catcher with a bait-pig on the following day; yet ~~a~~ search ~~was~~ made in the precise spot which subsequent events showed to be heavily populated by *G. medicorum*. *had been*

Late in the morning of 25th October tree trunks were searched and two *G. medicorum* taken. Next day 14 more specimens were taken, and three times as many could have been taken had it been realised how to catch them. On the following day 20 specimens were taken and 25 missed. The best catcher took 15 and missed 7 in 1½ hours' search. In all, 37 specimens were caught—20 males and 17 females; two of the females contained black-lobed larvae, and one mammalian blood.

In four days only one specimen attacked, although *G. medicorum* were all around; it was a female and gave a very painful bite below the knee.

Since *G. medicorum* is considered to be a rare species of tsetse about which virtually nothing is known, the following observations may be of interest.

The area investigated was within 100 yards of the Ogun river. The vegetation consisted of natural high forest closely underplanted with *Albizzia* sp. and *Bauhinia* sp., some of which attained a height of 20 to 30 feet; the former tree produces a fine feathery canopy. The bulk of the flies taken were resting on the trunks of saplings of these species; the thinnest saplings, about one inch in diameter, were the most favoured, although flies were seen on stems up to three inches in diameter. One specimen only was taken on the large trunk of a natural forest tree, and two specimens were taken on bamboo stems. Only dappled sunlight reaches the floor of these plantations, and herbaceous growth is largely suppressed. In places the saplings are so closely planted that it is difficult to squeeze between them (Pl. V, fig. 1).

With only one exception, the flies were all found resting head downwards on the shaded side of the trunk. Whereas the tip of the wings and abdomen rested on the bark, the anterior portion of the fly was raised considerably above the surface. When seen in profile in a reasonably good light, one could easily mistake *G. medicorum* for a large red ant (Pl. IV, fig. 1).

There would appear to be a considerable variation in the resting height. On the first day, the height above ground-level was recorded as being between 2½ to 5 feet; on the second day as 1½ to 7 feet, but on the third day the majority were resting between 5 and 8 feet up.

On two occasions, a pair of *G. medicorum* scuttling up and down the trunk were seen chasing each other and making small hops into the air and back again; presumably this was a preamble to copulation. Twice, copulation was observed by recently caught specimens within a pill box; the female sits beneath the male with her wings parted. One couple remained paired for two hours.

G. medicorum and *G. palpalis* were seen on one occasion resting together on the under side of a horizontally-slung creeper; this was the only instance observed of *G. medicorum* resting on a horizontal object.

To start with, we were greatly perplexed as to how to catch this species. Little difficulty was experienced in advancing the edge of the net until it touched the tree a few inches below the fly; but after the usual, very rapid upward sweep of the net had been made, it was found that, three times out of four, we had missed. One of us then conceived the possibility that the reactions of *G. medicorum* might be so slow that, instead of perceiving the rapidly approaching net in time to fly off the tree-trunk—thus permitting the fly's entry into the net bag—the insect might remain sitting until brushed off by the edge of the net when it would fall to the ground and be lost. Experiments soon showed that this conjecture was correct, as it was found that by carrying out the usual movements in slow-motion, and by making the upward sweep at a fraction of the usual speed, this species could readily be caught.

All the observations upon resting habits given above were made on sunny mornings between the hours of 10.30 a.m. and 12.45 p.m.; there had usually been a little rain in the night, and the forest was hot and steamy. Circumstances did not permit of an investigation to determine whether this species is active at dawn or dusk, or during the night.

There was a considerable amount of antelope spoor in the vicinity of the area described.

It must be emphasised that the natural vegetation in this locality has been greatly modified by the introduction of plantations under the high forest. The nearest natural counterpart would be young secondary forest during the stage when the initial thicket is being suppressed by emergent saplings: old farmland in which the forest giants had been spared would reproduce the high shade conditions found in this part of the reserve. Cocoa and cola plantations under high forest might also produce somewhat comparable conditions. These ideas are put forward because the findings suggest the possibility that this species may be far more widely distributed than the existing records show, and that attention directed to the searching of the trunks of young trees in the types of vegetation indicated might prove profitable.

Glossina fusca (Wlk.).

Odd specimens of this species had been taken in the past, and as previously mentioned, quite large numbers had been taken in the previous dry season: these were caught in the extensive, natural high forest on either side of the motor road. The forest is fairly open but the undergrowth, though low, is dense and not too easily penetrated.

This area was investigated on two mornings by 8 catchers, between the hours of 9 a.m. and 10.45 a.m.—when the forest was still cool and wet after rain which had fallen in the night. Screens and a bait-pig were quite ineffective and only *G. palpalis* was seen. Possibly *G. fusca* appears more readily to man in the dry season when it is hungrier.

On the second morning tree trunks were searched, but without success. Believing that failure might be due to the disturbance created in the undergrowth before we could approach to within adequate visual range, we moved on until we reached the first teak plantation, which was separated from the high forest by a narrow glade. Here, in the absence of undergrowth, search was easy and four specimens of *G. fusca* were taken by three of us in an hour; all were males and all were resting head downwards, with the forequarters higher than the hind quarters, on the shaded side of teak trees. Two were resting at 9 feet above ground level, one at 7 feet and one at 5 feet (Pl. IV, figs. 2 & 3).

There is no residual forest in this plantation to afford an upper canopy; it is a pure stand of closely planted teak with trees going up to about 40 feet, and with many smaller coppicing specimens with stems only about 1½ inches in diameter; there is no undergrowth, and the plantation is easily penetrated. Two specimens were taken well within the plantation and not just where it bordered the high forest. Since the teak trees will be quite leafless in the dry season, presumably they will not afford adequate shade later on in the year.

It may be mentioned that the long palps and *pronounced* dark blotch on the sterno-pleurae are the characteristics which readily serve to distinguish *G. fusca* from *G. medicorum* in the field. Owing to a considerable variation in *G. medicorum*, the other characteristics described by Newstead & others (1924) were not found to be very helpful.

Here then again was a case where failure to detect the presence of a species of tsetse would have resulted had tree trunks not been searched. Admittedly, we might have had very different results had we been able to make catches at dawn and dusk, but even so, with the "temperamental" members of the *fusca* group we might easily have failed to take any specimens in the few days at our disposal. Yet again, on tsetse surveys there is rarely time to spend many days in one place in order to take advantage of the brief, crepuscular hours.

It must be emphasised that in searching for these species extreme patience is needed; one may take half an hour to cover a few yards of forest if the trees are very close-spaced. One advances a step with the greatest caution so as not to set all the vegetation in motion by moving a creeper that links a number of saplings together; one pauses and methodically studies every trunk, first looking down each side for a tsetse in profile and then studying the bark; suddenly one realises that there is a tsetse, possibly within a foot or two of one's face. One always advances into the sun, because the flies will be on the shady side; this imposes a considerable eye strain.

Since we did not take the two species in the same places, *G. fusca* and *G. medicorum* would appear to have distinctive requirements and hence distinctive territories. It would be unwise to attempt to define the differences on such slender experience, but it may be noted that the *G. medicorum* were taken near the river.

Glossina longipalpis Wied.

At 10.30 a.m. on a sunny morning we moved down a ride through plantations of *Cassia* and *Bauhinia*, walking and stopping; but without being attacked by *G. longipalpis*. We then tried to attract this species to us in a very small clearing—a teak nursery—backing on the River Ogun. A month previously, and under similar conditions of time and weather, a fly-boy had taken 25 *G. longipalpis* within this clearing; all the specimens were males. During 45 minutes none of the 8 catchers saw *G. longipalpis* although screens were used; on the following day a bait-pig proved to be equally unsuccessful.

We then returned some 50 yards up the ride and started *searching*, and soon took a specimen sunning itself on a leaf. We now entered the adjacent plantations which consist of *Cassia* and *Bauhinia* saplings, and which in the absence of high shade support a short growth of grass and herbaceous plants.

In searching for this species one has to keep quiet, as success depends considerably on hearing. There is the distinctive whine of the fly as it approaches, followed by silence; by studying the leaves, twigs and sapling trunks, one can often pick out the fly. One should walk quietly, listening until one hears a following fly and then stop near some large-leaved shrub or tree trunk, hoping that the fly will alight on it, and so reduce the subsequent labour of searching many objects (Pl. V, fig. 3).

On the first day, screens seemed to help to advertise our presence, although only one fly actually settled on a screen; they would settle within a foot or two on the adjacent herbage. Seven flies were caught between 11.45 a.m. and 12.15 p.m., and about an equal number were missed; of the seven caught all were males.

On this first day no single specimen alighted on a man, so that the presence of *G. longipalpis* might easily have been overlooked if the technique described had not been adopted.

On the second day the same plantations were revisited between 10.45 a.m. and noon; 12 flies were taken, and again all were males. They were caught off leaves, twigs and sapling trunks—often in the sun. One specimen attacked a man and bit him on the ankle. No flies settled on the bait-pig.

We tried moving very slowly so as not to advertise our presence, and so to catch flies that were already at rest—but all such captures also turned out to be males. Possibly the females rest on the under side of leaves (Pl. V, fig. 2).

Out of 25 flies caught during four days only one was a female and only two attacked or even settled on man.

It would appear that under conditions where game is abundant *G. longipalpis* is very little interested in man, and that consequently its presence could easily be overlooked by those not used to the technique of listening, stopping, and looking.

Conclusions.

A short fly-survey has been described which demonstrates how the presence of two, and possibly three, species of tsetse might have been overlooked, had not recourse been made to the technique of searching for resting flies.

Some observations on the habits of *G. medicorum* are recorded, since this is a species about which very little is known.

References.

- KEAY, R. W. J. (1949). An outline of Nigerian vegetation. Government Printer, Lagos.
- NEWSTEAD, R., EVANS, A. N. & POTTS, W. H. (1924). Guide to the study of tsetse flies. London, Hodder & Stoughton.
-

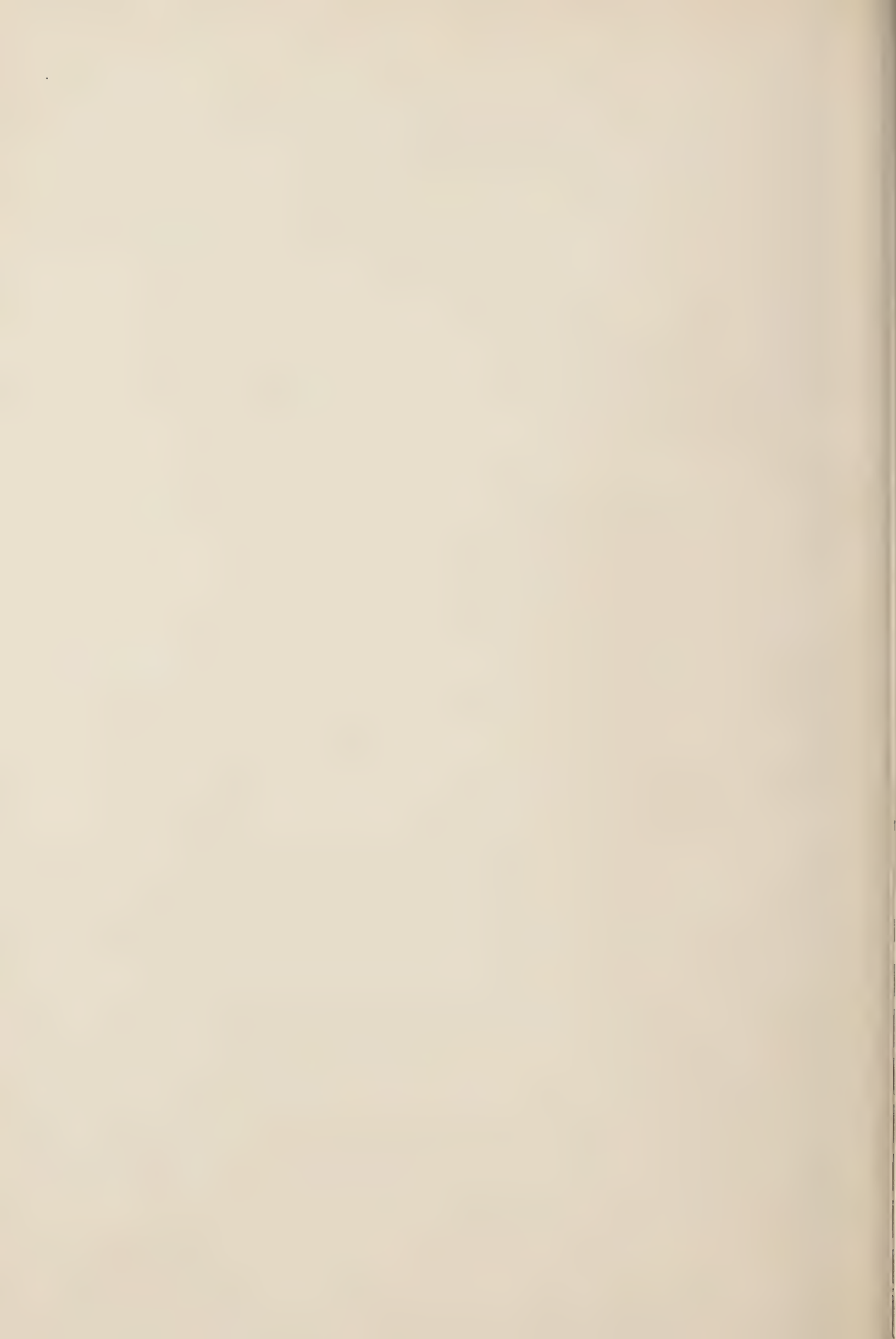




FIG. 1. View, up into canopy, of *G. medicorum* resting high up on a sapling. Note the characteristic resting position with head well raised above bark.



FIG. 2. A fly-boy, astride another man's shoulders, reaches up to catch *G. fusca* which is at rest some nine feet up on a teak sapling.



FIG. 3. Searching tree trunks for resting *G. fusca* in a teak plantation.





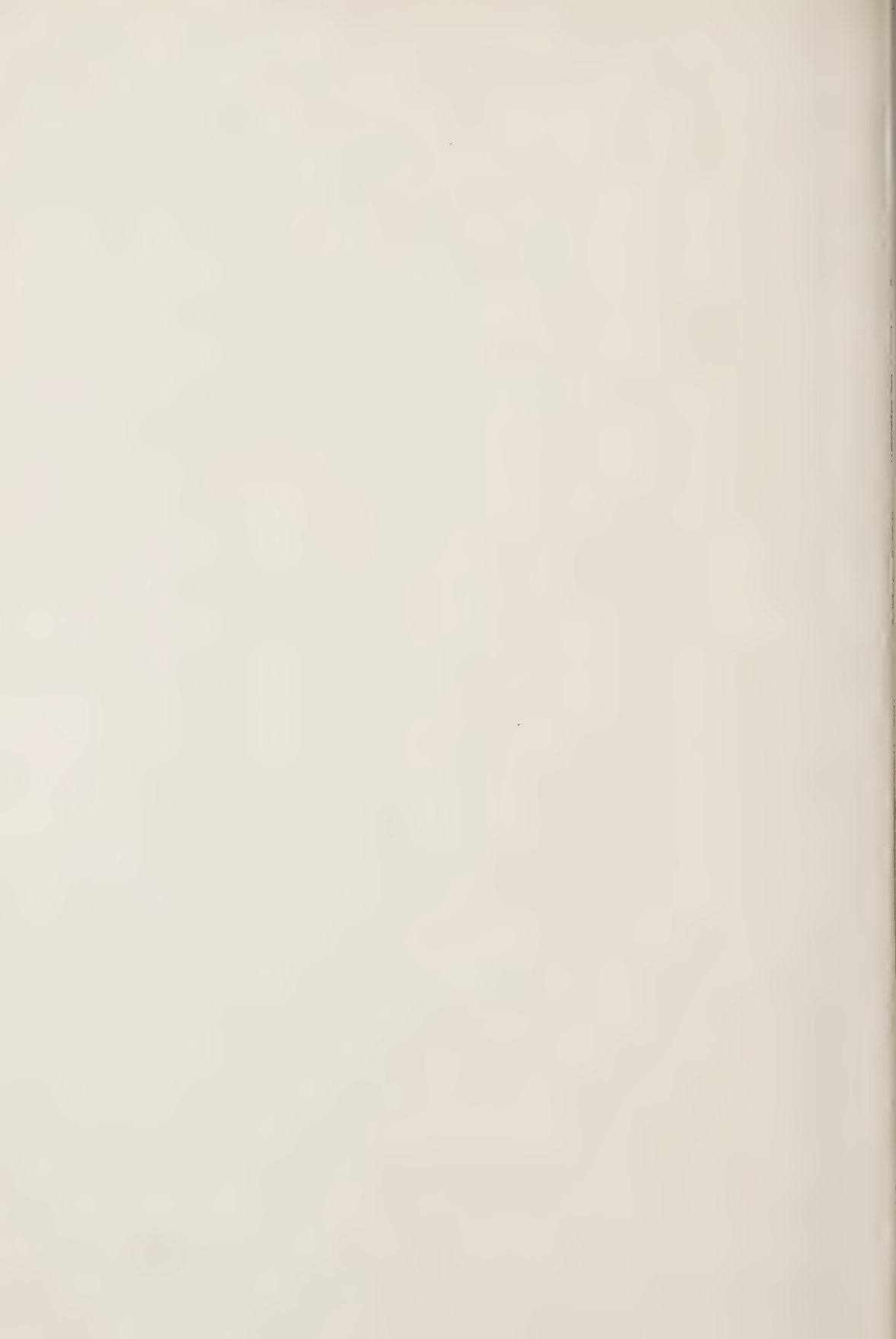
FIG. 1. *Albizia* plantation under high forest—the habitat of *G. medicorum*.
N.B. No grass grows beneath the dense canopy.



FIG. 2. *G. longipalpis* at rest on a sapling. A white helmet was held behind to afford a light background.



FIG. 3. Searching for *G. longipalpis* which may be at rest, or which may alight, on sapling trunks or leaves near the collector. N.B. The canopy within these *Cassia* and *Bauhinia* plantations does not suppress grass growth.



WET SEASON FRAYING OF WINGS OF TSETSE-FLIES, *GLOSSINA MORSITANS*.

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In two previous papers (Jackson, 1946, 1948), some results showing the fraying of the trailing edge of the wings of male tsetse-flies (*Glossina morsitans* Westwood) with the passage of time from emergence have been given. The data were obtained by allowing the flies to emerge for three days only from large numbers of pupae exposed in shade in the habitat of *G. sayneri* Austen or of *G. pallidipes* Austen, where *G. morsitans* was not normally present. All recaptures could thus be assigned to emergence within the three days during which the pupae were exposed, and, until the appearance of the second generation at upwards of 50 days, the flies caught were all of known age, and had been at large for various known periods.

These experiments had been done about the middle of the dry season in successive years and showed no material difference from each other. In testing for a possible difference in the early part of the rainy season (January, 1949) it proved difficult to obtain such large numbers of pupae as were used in the dry season, and the pupae were therefore exposed for five days instead of the usual three. On this occasion the habitat of *G. pallidipes* was used. Because of the small numbers of pupae available, only 48 recaptures of male *G. morsitans* were obtained, the oldest being 39 to 43 days from emergence.

In the comparative tables of wing fray in the dry and rainy seasons (Tables I and II), the time units are in 5-day groups, 1-5, 6-10, etc., and the amounts of wear on individual wings are expressed as the square root of the percentage missing from the linear hind margin, from tip to alula (Jackson, 1946). These square roots are then grouped as .0 to .4, .5 to .9, . . . 8.5 to 8.9.

TABLE I.
July 1945.

Root fray group	Time group													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1	20	7	13	4	—	1	—	—	—	—	—	—	—	45
2	—	—	—	—	—	—	—	—	—	—	—	—	—	0
3	5	9	13	9	4	1	2	—	—	—	—	—	—	43
4	1	2	1	5	6	1	—	—	—	—	—	—	—	16
5	1	3	7	9	15	3	—	—	2	—	—	—	—	40
6	—	1	2	1	7	4	2	1	—	—	—	—	—	18
7	—	—	1	4	9	2	1	4	—	—	—	—	—	21
8	—	—	1	2	8	6	4	1	—	—	—	—	—	22
9	—	—	1	2	2	2	3	6	3	—	—	—	—	19
10	—	—	—	1	1	3	6	1	3	—	—	—	—	15
11	—	—	—	—	—	2	3	3	1	1	1	—	—	11
12	—	—	—	—	—	—	4	4	2	1	—	—	—	11
13	—	—	—	—	—	—	—	4	—	—	2	1	—	7
14	—	—	—	—	—	—	—	—	3	1	1	1	1	7
15	—	—	—	—	—	—	—	—	4	1	—	1	1	7
16	—	—	—	—	—	—	—	1	—	—	—	1	—	2
17	—	—	—	—	—	—	—	—	—	—	—	1	—	1
18	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Total ...	27	22	39	37	52	25	25	26	18	4	4	5	2	286

TABLE II.
January 1949.

Root fray group	Time group									Total
	1	2	3	4	5	6	7	8	9	
1	5	—	4	—	—	—	—	—	—	9
2	—	—	—	—	—	—	—	—	—	0
3	1	2	6	3	—	1	—	—	—	13
4	—	—	2	1	1	—	—	—	—	4
5	—	—	1	1	—	1	—	—	—	3
6	—	—	—	2	—	1	—	—	—	3
7	—	—	—	—	—	—	—	2	—	2
8	—	—	—	—	1	1	1	—	—	3
9	—	—	—	—	—	—	2	1	—	3
10	—	—	—	—	—	—	1	—	—	1
11	—	—	—	—	1	—	—	—	1	2
12	—	—	—	—	—	1	—	—	—	1
13	—	—	—	—	—	1	—	1	—	2
14	—	—	—	—	—	—	—	1	—	1
15	—	—	—	—	—	—	—	1	—	1
Total ...	6	2	13	7	3	6	4	6	1	48

The regression coefficients are respectively 1.25 and 1.42 and there is no significant difference between them. In neither case is there any significant deviation from linear regression. There is thus no indication of any effect of season on the rate of fraying of the wings.

My colleague, Mr. A. T. Culwick, has pointed out that if, as is apparently the case, it is the square root of the percentage fray which is directly related to the time, then the brittleness of the trailing margin must also be directly related to the time: in other words the probability that any particular section of the trailing margin will wear away in any particular time-interval increases directly with the passage of time. Certainly the wings seem to become stiffer with age, and may become more brittle as they stiffen.

It might be thought that the fray-with-age formula might be affected by the law of diminishing returns, in that when any particular section has been worn away it cannot be frayed, on the margin at least, any more. The data do not give any indication that this is so, but comparatively few flies have very worn wings, and until this state is reached the law of diminishing returns would not have very much effect. Possibly also, when two small sections of the trailing margin near to each other have become worn, the part between them tends to drop out, or at least to wear more quickly, and if so would compensate for other sections which do not fray in any particular period because they have already done so.

Summary.

The wings of male *Glossina morsitans* appear to become frayed in the rainy season at about the same rate as in the dry season, and their brittleness seems directly related to age. The law of diminishing returns does not appear to apply, perhaps because of the weakening of portions of the trailing edge between indentations fairly near together.

References.

- JACKSON, C. H. N. (1946). Bull. ent. Res., **37**, pp. 291-299.
 JACKSON, C. H. N. (1948). Bull. ent. Res., **39**, pp. 441-451.

THE BIOLOGY AND EXTERNAL MORPHOLOGY OF THE LARVAE OF EPILACHNINAE (COLEOPTERA, COCCINELLIDAE).

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(Plate VI.)

The larvae of the subfamily EPILACHNINAE, like the adults, are herbivorous. They have a porcupine-like general appearance, as the body is usually clothed with long branched, spinous processes. The larvae, even of those species which are well known pests, have not hitherto been studied in much detail. On account of their rather uniform general appearance and the great complexity of the armature of the body-wall, the various species are not easily distinguished from one another, and the general descriptions that have appeared in papers dealing primarily with their biology and control measures are often insufficient for this purpose. Earlier workers who studied the larvae of Coccinellids were handicapped not only because the number of species of EPILACHNINAE available to them was very limited, but also because of the unsatisfactory state of the classification of the adult beetles and insufficient knowledge of their biology. Redtenbacher (1843) was apparently the first to draw attention to their phytophagous habits. Mulsant (1846), Candèze (1861) and Grandi (1913) each described one or two larvae of this sub-family. Böving (1917), Gage (1921), Strouhal (1927), and a few others who have studied the larvae of Coccinellids in general, have also described the structure of one or two species only, chiefly with a view to defining the larval characters of the subfamily as a whole. Few workers have hitherto attempted to distinguish more than two species or genera. The present study includes descriptions of 14 species belonging to six different genera of the subfamily from various parts of the world. Further, all the larval instars have been studied for three species and biological notes are given where possible.

Of the many points of interest concerning the family COCCINELLIDAE, those relating to their feeding habits have received considerable attention. The question as to whether the herbivorous habit is a primary or a secondary acquisition in the family will remain largely a matter of speculation until palaeontological evidence and greater knowledge of the relationship between the various groups (tribes and genera) of species are available. Observations on the feeding habits and the related structural differences are, however, discussed. The EPILACHNINAE comprise about one-sixth of the known species of the family. The adults present a great uniformity in external structure, with the result that nearly all of the known species have been placed in one genus, *Epilachna*. Of late, there has been a tendency to divide this subfamily into as many natural groups as possible, and for this purpose details of the external structures and of the genitalia are being increasingly employed. A study of the larvae, it is thought, will help not only in the identification of the species and genera but also in evolving a satisfactory and natural classification of the subfamily. In the following pages, the descriptions given are of the final instar larvae except where otherwise stated.

Besides the material in the British Museum (Natural History) and that collected by the author himself, collections of immature stages, as well as the adults in many cases, were received from Mr. J. C. M. Gardner, C.I.E., formerly of the Forest Research Institute, Dehra Dun (India), Mr. G. de Lotto of Eritrea, Mr. W. V. Harris of Uganda, Dr. E. A. Chapin of the U.S. National Museum, Washington, and Dr. F. van Emden,

of the Commonwealth Institute of Entomology, who also helped in other ways. Sincere thanks are due to all these gentlemen and also to Dr. W. J. Hall, Dr. T. H. C. Taylor and Dr. S. Maulik, for help of various kinds. To Dr. O. W. Richards the author is grateful for general supervision of the work. Mr. W. H. T. Tams very kindly prepared the photographs reproduced here. Most of the drawings were made with the aid of camera lucida by the author, except four (initialled A.S.) which were made by Mr. Arthur Smith.

General Description of the Larvae.

Body moderately elongate to oval, usually widest on the second or third abdominal segment and more narrowed posteriorly than towards the head; convex on the dorsal and rather flat to moderately convex on the ventral surface (Plate VI *a*).

Head relatively well developed, usually subrounded, sometimes slightly wider than long, directed at right angles to the longitudinal axis of the body, connected with the thorax by a moderately long membrane allowing limited movement in all directions; occipital foramen large and subrounded (fig. 15, *a*); vertex and genae convex, the front with an oblong-oval slight depression round a small central area. The epicranial suture, usually indicated by lighter coloration, consists of a coronal and two frontal sutures; the former extends from the base of the vertex to the middle of the distance between the base of the vertex and an imaginary transverse line joining the antennae; the frontal suture runs almost straight from the anterior end of the coronal suture to the base of the antenna; the clypeofrontal suture always present and usually distinctly indicated by strong sclerotisation of the frontal area. Ocelli, three on either side, dark, sub-conical, and arranged triangularly; one of them situated close and external to the base of the antennae, the other two at a little distance away towards the vertex and lying close together, usually of equal size,

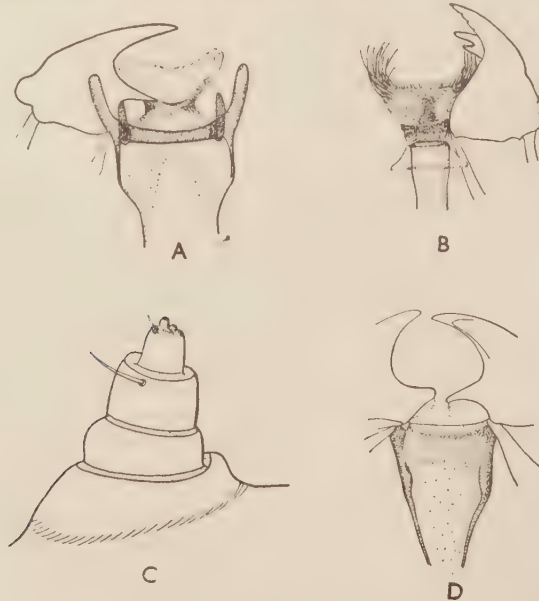


Fig. 1.—(a) *Brumus suturalis*, left mandible and hypopharyngeal sclerome of larva; (b) *Epilachna vigintioctopunctata*, right mandible and hypopharynx of first-instar larva; (c) *Coccidula scutellata*, antenna of larva; and (d) parts of mandibles and hypopharynx.

sometimes the inner one larger. Antennae, two- or three-segmented, usually long (two to three times as long as wide), but sometimes short (one-and-a-half times as long as wide), with the basal antacoria well developed and arising from a slightly elevated, circular part of the cranium forming the antennal socket. Clypeus usually well developed and demarcated by clypeofrontal suture, trapezoidal, narrowing anteriorly and bearing a few setae on the lateral margins. Labrum rectangular or transversely elliptical, variable in size in certain genera. The mandibles, which provide the most characteristic feature of the subfamily, are without basal teeth and have three to seven (or more) major teeth in the distal part; some of these teeth are denticulate on the inner margin. The hypopharyngeal sclerome* not sclerotised (fig. 1 *b*). The maxillae and labium different in different genera; maxillary palpus characteristically long and three-segmented.

Thorax usually increasing in diameter towards the abdomen; less convex on the dorsal surface than the abdomen, with distinct pleurotergal and pleurosternal folds. Prothorax longer than either of the other two thoracic segments; pronotum sclerotised, with usually three, sometimes two, pairs of scoli† along the anterior margin and a few chalazae‡ and setae on the rest of its surface. Mesothorax and metathorax similar in shape and in the armature of the body-wall, each with three pairs of scoli on the dorsolateral surface, the area round the bases of scoli being sclerotised; the scoli differ from those of pronotum in that they are on the transverse median line of the segment. There is a single pair of spiracles on the thorax; each spiracle is situated on the dorsal aspect of the anterolateral part (sometimes called the spiracular area) of the mesothorax, and has a circular opening and is surrounded by a more or less sclerotised or pigmented area; thoracic spiracles usually larger than those on the abdomen. There is considerable uniformity in the arrangement of scoli on the thoracic and abdominal segments. The three pairs of scoli on each segment are arranged symmetrically on either side of the median longitudinal line. The scoli nearest the mid-dorsal line is called dorsal scoli, the next is called the subdorsal scoli, and the third, situated farthest from the mid-dorsal line and usually on the lateral margin or on the dorsal aspect of the pleuron, is called the dorsolateral scoli. On the underside of the segment, when the setae are arranged in groups or strumae, there are usually three pairs of these called ventral, subventral and ventrolateral. On the underside of the thorax, however, only the ventral groups of setae or strumae are present and in certain species the prosternum has only a single struma instead of one on either side of the median longitudinal line of the segment. The legs are similar in structure on all three segments; the part of the segment immediately surrounding the coxa is sclerotised and bears a few long setae; legs with long and sparse setae, except on the inner side of tibiae where the setae are dense, long and often thickened at the apices, the claw with a quadrate or triangular basal tooth and narrowed, bent moderately to strongly and pointed distally.

Abdomen ten segmented; the first eight segments each with three pairs of scoli and a pair of spiracles on the dorsal surface and with one to three pairs of the groups of setae or strumae on the ventral surface (but sometimes there is only a single seta representing each group); some of the strumae fused with one another in certain species. The dorsal scoli much closer to the median longitudinal line than is the case on the mesothorax and metathorax in many species. Each spiracle situated

*A hypopharyngeal sclerome is also called a hypopharyngeal bridge; a strongly sclerotised transverse part (usually with branches at either end) (fig. 1) at the base of the floor of the hypopharynx and usually present in the carnivorous species of the family (fig. 1 *a*).

†A scoli is a process, usually long and branched, of the body-wall, bearing setae at the apices of the branches; in the EPILACHNINAE it has also been applied to cases where it has become very short or simplified in structure by reduction in the number of branches.

‡A chalaza is a pimple-like swelling of the body-wall bearing a seta at its apex.

§A struma is a moderately convex area of the body-wall bearing a few setae or chalazae or both.

antero-laterally relative to base of subdorsal scoli, but on the first segment the spiracle is a little more dorsal. The ninth segment usually with a semicircular tergum bearing a number of both short and long setae or a pair of strumae; tenth segment short, mostly membranous, sometimes with a few very short setae, usually directed downwards and not visible from above.

Relationship with other Coccinellids.

The family COCCINELLIDAE is divided into three subfamilies, EPILACHNINAE, COCCINELLINAE and TETRABRACHINAE (LITHOPHILINAE). The larvae of the last named subfamily are quite unknown although, as far as can be judged from the structure of their mandibles, the adults appear to be carnivorous. The subfamily COCCINELLINAE is about five times as large as the EPILACHNINAE and presents a greater variety of form and structure. With the exception of the tribe Psylloborini, which is fungivorous and is very closely allied to Coccinellini in structure, the larvae and adults of the COCCINELLINAE are carnivorous. Although relatively little is known about the larvae of the Coccinellids as a whole, certain attempts to fix the position of the family in the order Coleoptera and to trace the relationship between the various subfamilies and tribes on the larval characters, have been made by certain workers in the past. While the conclusions presented by Böving (1917) and Böving and Craighead (1931) are in general accord with those of other Coleopterists, such as Ganglbauer (1899), and Sharp and Muir (1912) who studied the adults, Gage (1921) and Strouhal (1927) arrived at quite different results. Böving, and Böving and Craighead, regard the family COCCINELLIDAE as belonging to the Cucujoidea and *Hyperaspis* larvae as representing a more generalised form of the family. Gage and Strouhal consider CHRYSOMELIDAE as the probable progenitors of the family and EPILACHNINAE as the more generalised form. Again, while Böving regards EPILACHNINAE as allied to the tribe Coccinellini, Gage regards it as being close to Chilacorini, Strouhal, however, considers that Chilacorini and Coccinellini have probably originated independently from the EPILACHNINAE.

These differences in conclusions are apparently due to the limited number of the larvae studied so far, and also to the fact that different authors have stressed different characters. Apart from being herbivorous, the larvae of CHRYSOMELIDAE and EPILACHNINAE differ a great deal both in structure and in their mode of feeding. The structural differences have been dealt with by Böving and by Böving and Craighead, and need not be repeated here. Attention may, however, be called to the differences in their feeding habits. The EPILACHNINAE scrape and compress the leaf tissue with their mandibles and imbibe the juices and softer tissues, rejecting the cellulose and other harder tissues of the leaf previous to ingestion. The long and usually dense setae on the galea and neighbouring parts help in holding the juices during the process of scraping. The excrement is passed out in fluid form, unlike that of Chrysomelids and phytophagous caterpillars, which usually ingest leaf tissue in bits and pass the excrement in solid lumps. These and similar observations were made by Howard (1941) for *Epilachna varivestis* larvae, and subsequently by others for several more species of EPILACHNINAE. Regarding the relationship between the latter and the Chilacorini, Gage and Strouhal appear to have laid great emphasis on their common characters in respect of the coronal suture and the scoli of the body wall. The coronal suture, which is always present in the EPILACHNINAE, exists in only three closely related genera—*Chilocorus* Leach, *Orcus* Mulsant and *Egius* Mulsant, in the CHILOCORINI. It is also present, though very much shorter, in *Ceratomegilla maculata* (De Geer) which is a member of the tribe COCCINELLINI. Similarly, there is great variation in the degree of development of scoli on the thorax in the various species and genera of CHILOCORINI, and even in COCCINELLINI the scoli are present in several species though not usually well developed. The 14 species of EPILACHNINAE studied here have shown that the scoli vary a great deal in size and

form in various genera ; in *Chnootriba* Chevrolat and *Merma* Weise, for example, the scoli are even shorter or have more reduced branches than in certain CHILCORINI and COCCINELLINI. It appears, therefore, that these characters are of little value for tracing the relationships between the various groups. Böving regarded EPILACHNINAE as being allied to COCCINELLINI on account of their similar general appearance, the position of the thoracic spiracles and the shape of the terga and pleurae on the thoracic segments. In the form of the hypopharyngeal sclerome and the structure of the mandibles, he regarded EPILACHNINAE as occupying a unique position in the family. The structure of the clypeofrontal region and of the antennae were also considered by him to be characteristic of the subfamily. The present studies show that while the mandibles and antennae present greater variety of structure than was the case in the four species examined by Böving, the importance of these characters remains undiminished. According to the classification based on the adults, the tribe COCCIDULINI is regarded as more generalised among the subfamily COCCINELLINAE. An examination of the larvae of *Coccidula scutellata* (Herbst), *C. rufa* (Herbst) and *Rhizobius litura* (F.), members of COCCIDULINI, revealed that the hypopharyngeal sclerome was not so strongly sclerotised (fig. 1 *d*) and did not have any branches at either end, unlike the known larvae of most other tribes of the subfamily ; thus, in this respect, the COCCIDULINI are closer to the EPILACHNINAE than to any other tribe. Maxillary palpi in the two are also long. The antennae of *Coccidula* (fig. 1 *c*) and *Rhizobius* are moderately long, narrowed distally, and composed of three clearly defined segments of nearly equal length. They differ from the very short antennae in most other tribes of COCCINELLINAE and from the rather long ones in the EPILACHNINAE. In most other characters of the head and of the armature of the body-wall the larvae of COCCIDULINI resemble the other known larvae of the subfamily. With our limited knowledge of the related forms, the question of the relationship with EPILACHNINAE may not be easily answered. It seems probable, however, that at least among the COCCINELLINAE it represents a more generalised form than either HYPERASPINI or CHILCORINI.

Key to the Genera.

1. Subdorsal scoli absent on pronotum ; scoli* long, some longer than width of body, with numerous long and short branches interspersed ; mandibles broad at base, with three blunt teeth.....*Afissa* Dieke
Subdorsal scoli present on pronotum ; scoli short or long but distinctly shorter than width of body, branches less numerous, the shorter ones being usually near the base ; mandibles broad or narrow at base but with more than three teeth..... 2
2. Claw without a quadrate basal tooth ; scoli very short, with short branches arising like a rosette from a conical base, and bearing equally short setae ; labrum very short, subrectangular ; galea elongate, strongly sclerotised on the inner margin.....*Chnootriba* Chevrolat *in* Dejean
Claw with a quadrate basal tooth ; scoli moderately long to long, branches not arising like a rosette ; labrum large, usually subrounded laterally ; galea oval or subrounded, not sclerotised on the inner margin..... 3
3. Ocelli of unequal size, the area round them of lighter colour ; scoli moderately long, with short branches bearing very long setae (nearly four times the length of the branch)..... 4
Ocelli of equal size, the area round them relatively dark ; scoli long to moderately long, with long or short branches but with shorter setae..... 5

*Except where otherwise stated, the term "scoli" in the key refers to the dorsal and subdorsal scoli of the abdomen, especially of the first four or five segments.

4. Head with the median anterior part of the front broadly triangular, strongly sclerotised, the part posterior to the sclerotised area with three pairs of rather short setae; mandibles elongate, much narrowed distally, each with one large, apical and three short subapical teeth.....*Cynegetis* Chevrolat *in* Dejean

Head with the median anterior part of the front not broadly triangular, usually with six pairs of short setae crowded together; mandibles very broad at base, not much elongated or narrowed distally, each with two large apical and three moderately large to small, subapical teeth.....*Subcoccinella* Guérin-Ménéville

5. Mandibles broad at base, not much narrowed distally, with seven teeth, four of which are large and sharp; clypeus mostly membranous, larger than labrum which is expanded laterally at the anterior angles; galea subrounded with very short setae; scoli long with short branches and setae; dorsal scoli on the seventh and eighth abdominal segments equally long.....*Merma* Weise

Mandibles elongate and much narrowed distally, with not more than five teeth, two of which are large; clypeus mostly sclerotised, shorter than labrum which is not expanded distally; galea oval, with long, dense setae; scoli long, with usually long branches bearing much shorter setae; when the branches and setae are equally short, the former arise bilaterally from the main-stem, dorsal abdominal scoli on the eighth segment much shorter than on the seventh.....*Epilachna* Chevrolat *in* Dejean

Genus *EPILACHNA* Chevrolat *in* Dejean.

The larvae of the genus *Epilachna*, as at present constituted, show a great variety of structure. This is probably due to the fact that earlier workers have been misled into placing all the species concerned into one genus by the uniformity that the adult beetles display in certain external characters, including general appearance. In recent years, however, a more detailed study of their external structure and genitalia has resulted in the species being arranged into new groups, and some being separated into new genera. The present study of the larvae lends some support to this arrangement. The genus *Afissa*, for example, which was separated from *Epilachna* by Dieke in 1947, presents larval characters that are very distinct from those of *E. borealis* (F.), the type of the genus. The genus *Chnootriba* Chevrolat (*in* Dejean) which was sunk under *Epilachna* by Weise (1898) but has finally been recognised as valid by Mader (1941), also has distinct larval characters. From the accompanying descriptions and key to the species of *Epilachna*, it will be observed that the characters separating the species from one another within a group are much less pronounced than those distinguishing one group of species from another. This is equally true of the adult beetles, provided that the characters employed are more reliable than those, such as coloration and markings, that have been used hitherto.

Key to the fourth-instar larvae of Epilachna described in this paper.

1. Body yellowish, most of the scoli with the stem and spines piceous and the branches partly or wholly piceous; antenna long, with third segment indistinct; subdorsal scoli on pronotum with two or three very short setae, one of which is borne on a small branch; dorsal and dorsolateral scoli on pronotum not much broader towards the base than most other scoli...(borealis group) 2

Body yellowish, brown or piceous with scoli yellowish to dark brown; antenna usually long with the third segment distinct; when short, the third segment indistinct; subdorsal scoli simple or branched, the dorsal and dorsolateral scoli on pronotum much broader towards the base than other scoli.....3

2. Mandibles rather elongate, their length much greater than their width at the base and narrowed distally, the two large subapical teeth united at base ; scoli with the main stem and usually the basal part of branches piceous ; dorsal scoli on first seven abdominal segments with about eight long branches , ventral strumae each with three or four setae, those on the seventh segment lying close to the subventral strumae, and those on the eighth being confluent with them (America).....*borealis* (F.)
Mandibles broad at the base, only a little longer than broad and narrowed distally, the two large subapical teeth separate ; scoli with the main stem and branches piceous, the dorsal scoli on the first seven abdominal segments with about twelve long branches ; ventral strumae each with about six setae, ventral and subventral strumae separate on the seventh segment and close to each other on the eighth (America).....*varivestis* Muls.
3. Head with a few short setae, usually restricted to vertex and anterior part of front, other setae moderately long and sparse ; antennae long, third segment distinct, second segment about twice as long as wide, with the seta below the apical margin4
Head with numerous short setae, intermixed all over its surface with long setae ; antennae short, third segment indistinct, second segment only slightly longer than wide, with the seta at the apical margin (Europe, N. Africa).....*argus* (Geoffr.)
4. Scoli with the branches arising all round the stem ; abdomen with the dorsal scoli equal in length to or slightly shorter than the subdorsal ones ; ventral surface with the setae arranged in groups or strumae.....5
Scoli with branches arising usually bilaterally from the main stem ; abdomen with the dorsal scoli much shorter than the subdorsal ones ; ventral surface without strumae or distinct groups, the setae being irregularly dispersed.....(*eusema* group) 8
5. Scoli moderately long, becoming distinctly broader towards the base, branches close, and (except for the apical setae) not bearing short, very thin setae ; meso- and metanotum with the bases of dorsal and subdorsal scoli of the same side not very close together.....6
Scoli long, only slightly broader towards the base, branches sparse and each bearing (in addition to the apical setae) two or three short and very thin setae ; meso- and metanotum with the bases of dorsal and subdorsal scoli of the same side very close together.....(*vigintioctopunctata* group)
6. Subdorsal scoli on pronotum usually with two branches ; dorsal surface or body-wall mostly piceous with the scoli and the area round their bases lighter (brownish) ; ventral and subventral strumae separate on the seventh segment and close to each other on the eighth (Africa).....*hirta* (Thnb.)
Subdorsal scoli on pronotum unbranched ; body mostly light yellow, with scoli and the area round their bases usually light brown or brown ; ventral and subventral strumae contiguous on both the seventh and eighth segments (Africa, Europe, Asia)*chrysomelina* subsp. *orientalis* Zimm.
7. Scoli as long as the width of the head, with twelve rather close branches ; strumae on the underside with the bases not strongly sclerotised or pigmented brown ; prosternum with a single struma in the middle ; ventral and subventral strumae on eighth segment fused (Asia)..... *vigintioctopunctata* (F.)
Scoli distinctly longer than the width of the head, with twelve sparse branches ; strumae on the underside strongly sclerotised and pigmented brown ; prosternum with a pair of strumae ; ventral and subventral strumae on eighth abdominal segment separate (Asia).....*dentulata* Dieke

8. Body lightly yellow with the part of the abdominal tergites between the dorsal pair of scoli brown to dark brown ; scoli with short branches bearing short apical setae *eusema* (Ws.)
- Body dark brown to piceous on the dorsal surface, with the scoli whitish except for the black, short, apical setae borne by the long to moderately long branches *flavofasciata* (Lap.)

***Epilachna borealis* (Fabricius) (Figs. 2 & 3).**

Epilachna borealis is of interest from both the economic and the taxonomic point of view ; in Central and North America it is a pest of cucurbitaceous plants. It is also the type of the genus *Epilachna*. A morphological description of the larva was given by Gage (1921) who also generalised the characters of the subfamily EPILACHNINAE.

Body (fig. 2) elongate oval, slightly narrower posteriorly, widest at the second abdominal segment. Fourth (last) instar larva 6.5–7.0 mm. long and 2.8–3.0 mm. wide across the second abdominal segment, excluding scoli which are about 1.75 mm. long. General colour of the body light yellow excepting the dark or light brown markings on the head, scoli and legs.

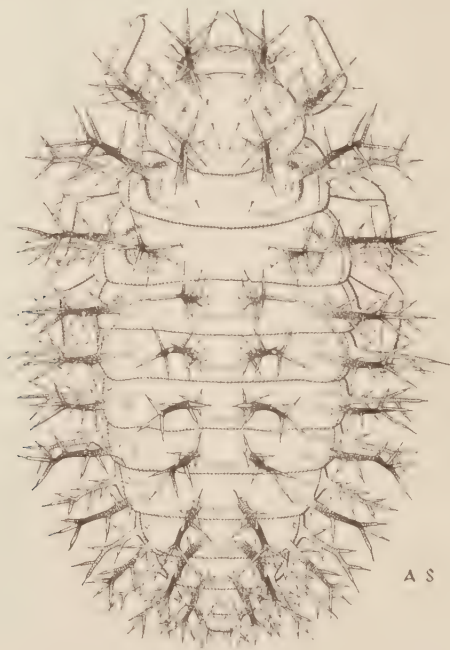


Fig. 2.—Larva of *Epilachna borealis*.

Head (fig. 3*b*) subrounded, a little narrower than prothorax, vertex and genae with brown to dark brown markings ; area round ocelli especially dark ; front rather lightly coloured except in and around a shallow, oval depression between the frontal sutures. Epicranial suture with both the coronal and frontal sutures lighter in colour, antero-clypeal margin distinctly sclerotised and marked dark brown. Setae moderately long, rather sparsely distributed, as shown in fig. 3*b*. Ocelli, three on either side, conical, piceous, of equal size, arranged triangularly, the two towards vertex much nearer each other than to the third which is situated near the

antennal socket. Antenna (fig. 3 *d*) with first segment slightly broader than long; second a little narrower than the first, slightly narrowing apically, nearly $2\frac{1}{2}$ times as long as wide, and bearing a long seta at two-thirds its length and a moderately short and conical sensilla near its apex; third segment light, rather indistinct, disc-like, and with a number of very short, conical sensillae. Clypeus (fig. 3 *b*) transverse, slightly narrower anteriorly, nearly four times as long as broad and bearing six or eight, short setae. Labrum slightly narrower at base, a little longer than clypeus; mandible (fig. 3 *e*) a little less than twice as long as wide, basal area plain, distally

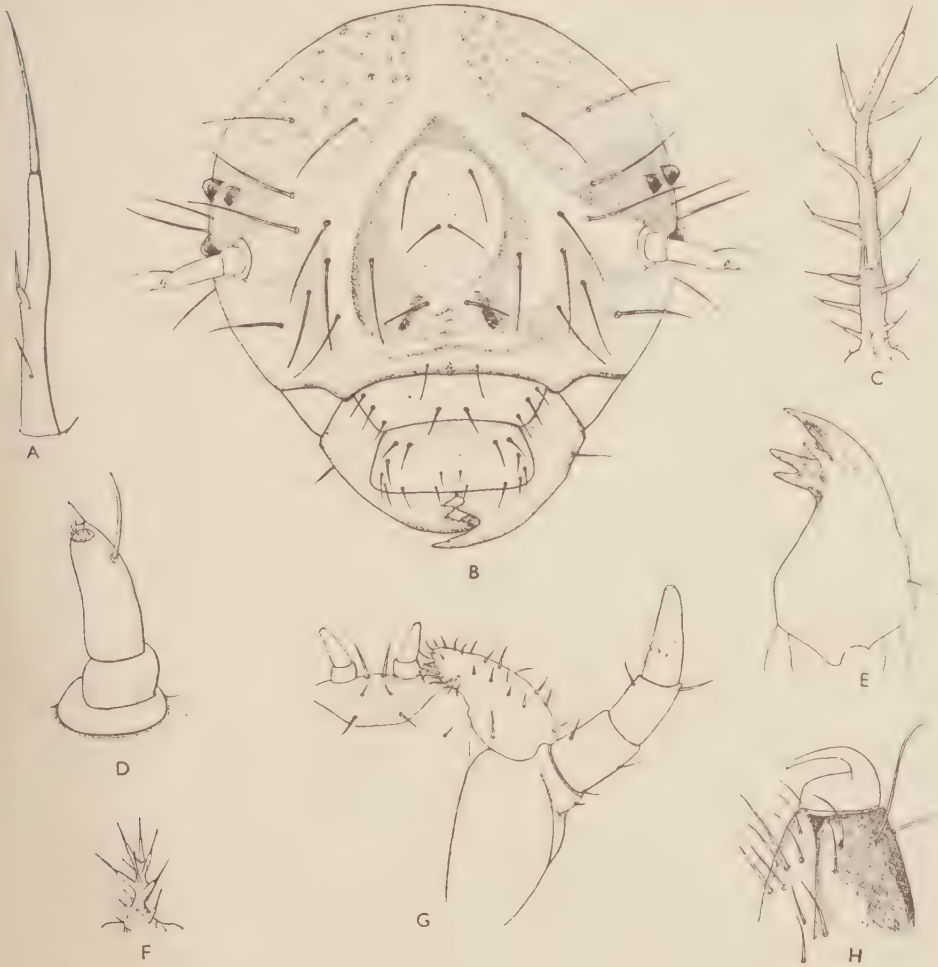


Fig. 3.—Larva of *Epilachna borealis*: (a) subdorsal scolus of pronotum; (b) head; (c) subdorsal scolus of second abdominal segment; (d) antenna; (e) mandible; (f) dorsolateral scolus of fifth abdominal segment; (g) maxilla and labium; (h) claw.

with four large and several small teeth, the large ones being weakly denticulate on the inner margin; maxilla (fig. 3 *g*) with galea subtriangular, rounded at apex, with with four large and several small teeth, the large ones being weakly denticulate on sparsely placed, short setae; palpus with apical segment filiform, narrower but slightly longer than either of the other two; labium oblong, membranous excepting

the rather weakly sclerotised area near bases of palpi, the latter short, with apical (second) segment elongate, cylindrical; mentum with two or three pairs of short setae in the middle.

Thorax gradually increasing in width, metathorax becoming as wide as first abdominal segment. Prothorax transverse, two-thirds as long as wide, pronotum with dorsal scoli arising near anterior margin, directed upwards and slightly forwards, brown to dark brown on main stem and light brown on branches, a little longer than the length of pronotum, and slightly tapering, with about 16 branches arising from all round main stem, but rather irregularly, and varying in length from short tubercles near the base to as long as two-thirds the entire length of main stem; usually three or four of the long branches arising from near the apex, six or seven long to moderately long ones arranged sparsely in the median half of the main stem and the remainder, moderately long to short, arising from near the base; apical seta of a branch usually brown, stout and nearly two-fifths the length of its branch. Subdorsal scoli (fig. 3*a*), on pronotum lighter, half as long as the latter and comprising a long filiform process with a long apical seta, a short branch, with a short seta, usually situated near middle of the scoli, and one or two short, simple setae situated between middle and the base. Dorsolateral scoli similar to dorsal scoli, arising a short distance from anterolateral margin of pronotum and pointing anterolaterally. Besides a pair of short chazae situated near centre of pronotum, there are about a dozen similar chazae arranged almost in a row along its posterior margin. Meso- and metathorax similar to each other, each shorter but wider than prothorax, nearly four times as wide as long, and with scoli on tergum similar to dorsal or dorsolateral scoli on prothorax. Dorsal scoli as far from the median longitudinal line as from lateral margin of tergum and placed very close to subdorsal scoli which is very similar to it. The areas of tergum round the bases of these scoli, and especially that situated laterally and posteriorly to the base of subdorsal scoli, heavily sclerotised and pigmented. Subdorsal scoli directed upwards and slightly laterally; dorsolateral scoli directed laterally. On the underside each struma has two to four short setae; prosternum with a single struma in the middle, meso- and metasternum, each with a pair of strumae. Legs: trochanter and femur together slightly longer than tibia, the latter dark brown on outside towards apex and as usual with dense and long setae on inner side; claw (fig. 3*b*) with a subquadrate basal tooth, distal part, narrow, pointed and subrounded near its base.

Abdomen: Dorsal scoli situated rather close to the median longitudinal line and the pair on each segment having a common, oval, sclerotised area round their bases; on first seven segments equal in size to those on metathorax, each with about twelve, long branches; on eighth segment short and lighter in colour and usually with eight rather short branches. Subdorsal scoli (fig. 3*c*) in line with the corresponding scoli on metathorax and likewise directed dorso-laterally except on the last three segments, where they tend to point rather posteriorly; on first seven segments, subdorsal scoli similar to those on metathorax in size, branching and coloration; on eighth they are much shorter, about two-thirds the length of dorsal scoli on the same segment, and with fewer setae. Dorsolateral scoli directed laterally, similar in size, general structure and coloration to the other scoli on first four segments but decreasing rather rapidly in size and number of branches (fig. 3*f* of fifth segment) on the succeeding four segments until, on the eighth, the dorsolateral scoli is merely a conical process with six to eight short spines, two or three of which are borne on small tubercles; dorsolateral scoli on sixth to eighth segments usually without brown coloration. The tergum of ninth segment semicircular, strongly sclerotised with light brown markings and about ten, short to moderately long, simple setae usually near its external margin. Ventral surface usually with subrounded to oval, rather lightly pigmented strumae. Ventral pair of strumae on first six segments distinct, each comprising 3-4 short setae usually in a transverse row; on the seventh

similar but situated by the side of subventral struma and on the following segment confluent with subventral strumae. The latter on the first two segments similar to ventral strumae of the same segment ; on third to seventh segments, each comprise four or five short to moderately long setae arising from small tubercles ; on eighth segment subventral and ventral strumae contiguous, with a total of six to eight setae. Ventrolateral strumae distinct from the rest on first eight segments ; on first segment similar to the ventral, on second to eighth segments, each comprising five or six setae, two or three of which arise from short, pimple-like projections. On ninth segment setae not arranged in separate groups, rather short, and usually not more than eight in all. Tenth segment membranous, with two or three very short setae on either side.

Material examined : Ten larvae (several pupae and adults) lent by Dr. E. A. Chapin of U.S. National Museum, Washington : six from Creton, Connecticut, on squash, 27.viii.43 and four from Quincy, Florida, on cushaws (*Cucurbita moschata*), 9.viii.44 (L.M. May).

***Epilachna varivestis* Mulsant (Fig. 4).**

Epilachna varivestis, commonly known as the Mexican bean beetle, is a pest of beans in Central America and the United States. The larva is very similar in general appearance to that of *E. borealis* from which it differs in a few structural details only.

Body similar in outline and size to that of *E. borealis* ; general colour light yellow, parts of head, tergites, legs and the majority of scoli dark brown to piceous.

Head similar to that of *borealis* in general shape ; but with the brown markings on vertex more diffused, genae and neighbouring parts of the front as dark as the area round ocelli. Setae moderately long and sparse and arranged as in *borealis*. Ocelli and antennae (fig. 4*a, b*) also similar to those in *borealis*. Clypeus very broad at base with the basal angles very acute, the anterior margin being much shorter than the posterior. Mandibles (fig. 4*c*) different from those of *borealis*, broad at base, slightly longer than broad, much narrowed in the distal half, with three large and two or three small teeth ; apical large teeth denticulate on the inner margin ; other mouth-parts as in *borealis*.

Thorax similar to that of *borealis* in general outline and in the arrangement of scoli, the latter, however, differing in the arrangement of branches and coloration. Prothorax with the tergum rather strongly sclerotised, dark brown especially along the posterior margin which bears a row of fairly closely placed chalazae. Dorsal scolus a little longer than the length of the tergum, slightly broader towards the base, with the main stem brown to dark brown, bearing about seventeen long to moderately long branches arising irregularly from all round its surface ; five branches near the apex dark brown, the rest uniformly coloured brown to light brown ; setae varying between two-thirds to two-fifths the length of the branches bearing them. Subdorsal scolus (fig. 4*e*) variable, a little shorter than the dorsal scolus, with a long seta at the apex, and two or three short ones (sometimes one of them arising from a short branch) at two-thirds to one-third the length of the main stem. Dorsolateral scolus slightly longer than the dorsal scolus, with about eighteen branches, as in the latter. Meso- and metathorax with the arrangement of scoli as in *E. borealis*, but different in coloration, each scolus (fig. 4*g*) with the main stem and the branches, dark brown except the few towards the base, which are lighter. Dorsolateral scolus with the main stem and branches light brown, but otherwise like those of the other scoli. Differs from *borealis* in structure of strumae on the underside ; prosternum with a pair of ventral strumae, each comprising three very short and closely placed setae on a small sclerotised area near the longitudinal median line ; on meso- and metasternum the strumae are as on the prosternum but with longer setae. Legs

similar to those in *borealis*, but with the distal part of claw (fig. 4*d*) acutely bent near the basal tooth and not subrounded as in the latter species.

Abdomen: Dorsal scoli on first seven segments of almost equal length; narrower at base than those on metathorax, with the main stem dark brown from a little above the base to the apex, branches and setae throughout of the same colour as the stem or slightly lighter; usually with a dozen rather long and about four short branches (the latter near the base), each branch bearing at its apex a short, stout seta usually equal to one-fourth the length of the branch, but in the case of short branches the setae not proportionately reduced. Dorsal scoli on eighth segment nearly half the length of that on the first, of light colour, with ten short branches. Subdorsal scoli on the first six segments nearly equal in size to dorsal scoli of the same segment, each with the area round its base heavily sclerotised, dark brown and emarginate on the side towards the centre of the segment, on the seventh and eighth segments shorter, usually lightly coloured, and each with eight to ten branches

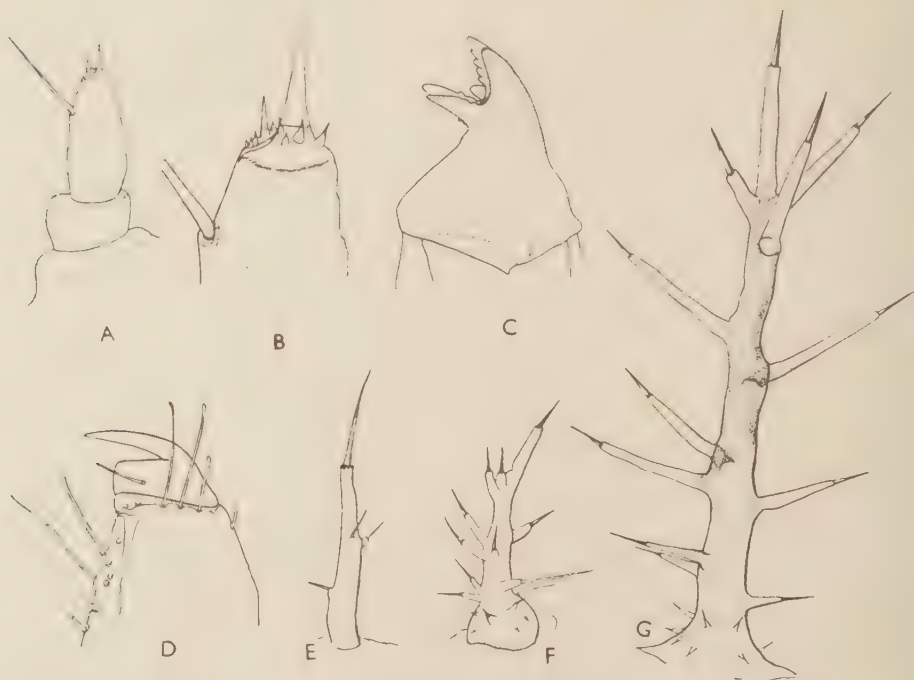


Fig. 4.—Larva of *Epilachna varivestis*: (a) antenna; (b) apex of antenna much enlarged; (c) mandible; (d) claw; (e) subdorsal scoli of pronotum; (f) dorsolateral scoli of fifth abdominal segment; (g) subdorsal scoli of second abdominal segment.

which are much shorter on those of the eighth than on those of the seventh. Dorsolateral scoli directed laterally, similar in size, general structure and coloration to the other scoli on the first four segments but decreasing in length and in the number of branches in each succeeding segment (fig. 4*f*, of fifth segment), and without heavily sclerotised areas round their bases; the scoli on the sixth segment with six short branches; that on the eighth with two short chazae and four short setae only. The tergum of the ninth segment semicircular, sclerotised, dark brown and with a dozen short setae, mainly along the external margin. The strumae on the ventral surface larger, more distinct and darker, being more strongly sclerotised than

those of *borealis*. Ventral strumae on first segment each represented by two short, rather transversely placed setae, on the second to seventh each ventral struma consisting of about six short setae, rather transverse oval; on the eighth similar to that on the seventh but lying close to the subventral struma. Subventral strumae on the first segment absent, on the second, struma with three short setae, but on the six succeeding segments with the number of setae increasing to about six. Ventrolateral struma on the first segment represented by a single short seta, on the second by one long and three to five short setae, and on the next three segments the strumae similar to those on the second but each with two or three long chalazae; on the sixth to eighth segments similar but smaller. On the ninth segment the setae are not grouped, but are distributed along the posterior margin and are about 18 in number. The tenth segment is devoid of setae ventrally.

Remarks.—The branches of scoli are uniformly pigmented either brown or piceous unlike those of *borealis* which are lighter towards the apices. The strumae are more distinct than in the latter species and also differ in their number and position on the prosternum and sternum of the eighth abdominal segment. The mandibles and claws show differences in structure as described above.

Material examined: One larva—same as described by Gorham (1898); 12 larvae from Berwyn, Maryland, 4.vii.1928 (*A. B. Gahan*).

Epilachna argus (Geoffroy, in Fourcroy) (Fig. 5).

Epilachna argus occurs in Central and Southern Europe and in North Africa. It attacks cucurbitaceous plants but is not reported to do serious damage. Brief descriptions of the larvae have been given by Mulsant (1846), Candèze (in Chapuis & Candèze, 1853) and Doebner (1862) but these are insufficient to distinguish it from other species. Schmidt (1922) also described the larva but this publication has not been available to the writer.

Body elongate oval, the final instar larva 10 mm. long, and 3.5 mm. wide; of variable colour, being brown to dark brown on the head, pronotum, the main stem of scoli and all or some of its branches, the openings of spiracles, the area round the bases of scoli, greater part of legs, and the bases of ventral setae; the remainder of the body light yellow.

Head (fig. 5*b*) subrounded; brown, with irregular, small darker patches on the vertex and dark brown area around the ocelli, the epicranial suture light brown. Setae very short to long, numerous, crowded, and arranged as shown in the figure; in addition, five or six moderately long setae present on the lower surface of genae. Ocelli, three on either side, of nearly equal size, black and arranged triangularly; the two towards the vertex close to each other and at a little distance from the third which lies close to and outside antennal socket. Antenna (fig. 5*a*) comparatively short, the first segment much broader than long, the second slightly narrower and a little longer than its diameter, with a moderately long seta and an elongate conical sensilla near its apical margin; the third small, indistinct, disc-like, lighter in colour, and bearing a few short sensillae. Clypeus two-and-a-half times as wide as long, with the basal half rather dark brown. Mouth-parts similar to those of *E. chrysomelina*.

Thorax distinctly increasing in diameter towards the abdomen. The arrangement of scoli on thorax and abdomen corresponds to that in *E. chrysomelina*. Prothorax half as long as wide, with the pronotum transverse oval, narrower posteriorly, strongly sclerotised and with irregular dark patches and a few shallow depressions on either side. Dorsal scolus (fig. 5*i*) a little shorter than length of pronotum, much broader at the base, with about 15 branches arising irregularly from all round its surface; branches moderately long to long, the apical two or three branches rather dark brown and with the setae dark and nearly as long as their branches; the

remaining branches brown or of very light colour, with the dark apical seta half to one-third as long as the branch bearing it; some branches occasionally with a very short seta in the middle. Subdorsal scolus of one side usually different from the corresponding scolus on the other side in the same example, varying in colour from light to dark brown; the one on the left side (fig. 5 *d*) of the pronotum like a large branch of the dorsal scolus, equal to about two-thirds the length of the latter, bearing an equally long seta at its apex and two or three short setae, arising from short tubercles situated near the base or the middle; that on the right side of the pronotum similar but with an additional (fig. 5 *c*), moderately long branch situated in the middle and bearing a fairly long, apical seta. Dorsolateral scolus similar to dorsal scolus. The centre of the tergum with a pair of moderately long chalazae and the posterior margin with about twelve, rather short chalazae and simple setae and metathorax with dorsal and subdorsal scoli longer than the dorsal ones on prothorax; each scolus with about fifteen long branches bearing short, brown, apical spines, usually not longer than one-third the length of the branches bearing them; the main stem and the apical three or four branches darker in colour. Dorsolateral scolus (fig. 5 *j*) similar to the other scoli on the segment but with a separate dark brown area round its base. Underside with dark brown strumae, the prosternum

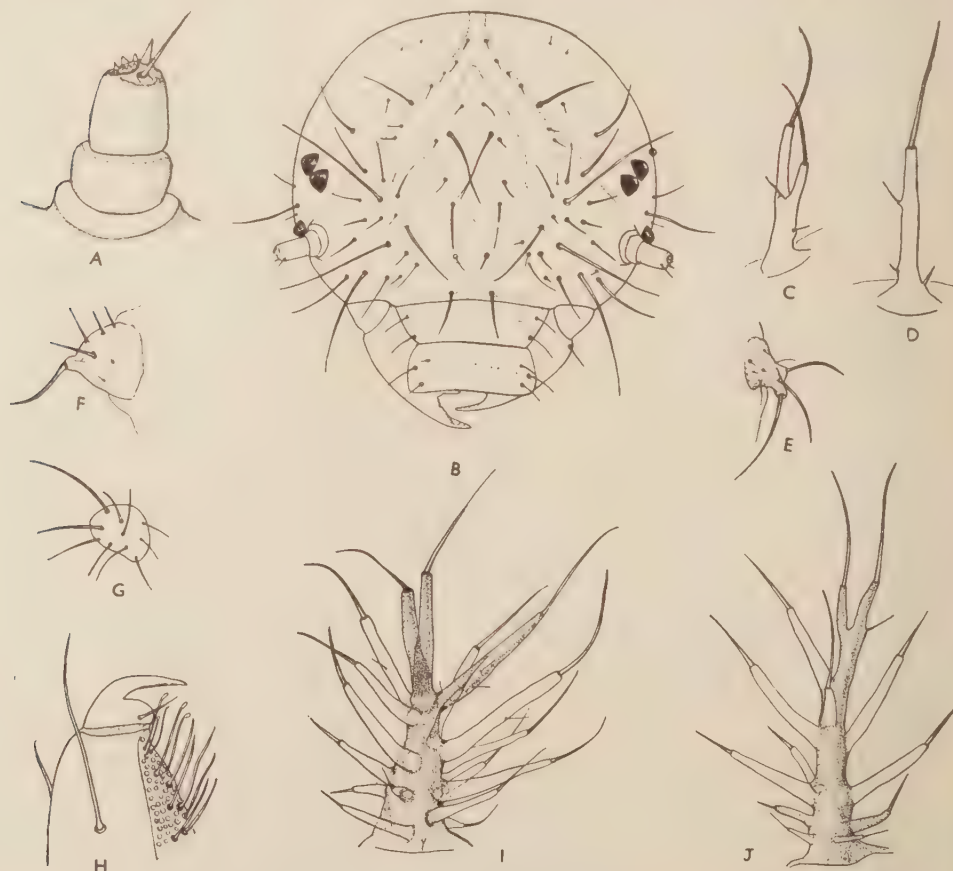


Fig. 5.—Larva of *Epilachna argus*: (a) antenna; (b) head; (c, d) subdorsal scoli of pronotum; (e) dorsolateral scolus of eighth abdominal segment; (f) ventrolateral struma of third abdominal segment; (g) the same of eighth abdominal segment (h) claw; (i) dorsal scolus of pronotum; (j) the same of mesonotum.

having one central struma with eight to ten setae ; on meso- and metasternum the strumae are separated but close together and each has about six short to moderately long setae. Legs as in *E. chrysomelina* but with distal part of claw (fig. 5 h) acutely bent nearer the basal tooth.

Abdomen : The pair of dorsal scoli on each segment, surrounded by a common, transverse oval to rectangular, sclerotised and darkly pigmented area ; scoli similar to those on metathorax in coloration and branching but slightly narrower at base, shorter and usually with 12 branches bearing short setae except for those on the eighth segment which have rather short branches but long and very light setae. Subdorsal scoli on the first seven similar to dorsal scoli but with the dark area round the base of each, subquadrate except for a short notch on the margin towards the centre of the segment ; on the eighth segment similar to dorsal scoli of that segment, nearly two-thirds as long and each with about eight branches. Dorsolateral scoli similar to the others on the first four segments but gradually decreasing in size on the next four segments, with apical setae of branches, however, becoming longer and lighter in colour ; on the eighth (fig. 5 e) being only a conical projection with a short branch and a number of moderately long to short setae. Ninth tergite semicircular, sclerotised, brown, with a few short setae in the middle and a row of moderately long ones at the posterior margin. Tenth segment with a small lightly sclerotised, brown area on either side, and bearing four very short setae. Ventral surface with usually dark brown strumae ; ventral strumae on first segment with two or three very short setae, on the second with four, usually subtransversely arranged, on the third to fifth each with eight to ten setae but then decreasing gradually in number to about three on the eighth segment. Subventral strumae usually absent on the first segment ; each consists of one long and two short setae on the second ; of six to ten moderately long and closely placed setae on each segment from the third to sixth, and of about five setae on the seventh and eighth. Unlike those of *E. chrysomelina*, not contiguous with the ventral struma on these two segments. Ventrolateral struma on the first segment represented by a single, usually brown chalaza with a long seta, on the second increases in size and has one chalaza and two short setae ; on the third with three long and usually four moderately long setae ; on the fourth to sixth segments the setae become longer and increase in number to about ten or twelve in each struma ; on the seventh and eighth the number is reduced to seven and five, respectively. Ninth segment with two strumae on either side, each consisting of three to five short, brown setae. No setae present ventrally on the tenth segment.

Material examined : Three larvae from Central Europe (*Verhoeff*) and four from North Algeria, 25.v.1913.

Remarks : By its head having numerous short setae intermixed all over its surface with long setae and by the short antennae, this species is very distinct from the other species. The structure of the adult beetles, especially of the male genitalia, also supports this.

***Epilachna chrysomelina* subsp. *orientalis* Zimm. (Fig. 6).**

Epilachna chrysomelina (F.) occurs throughout Africa, southern and middle Europe and central and southern Asia. It is divisible into several subspecies ; according to Zimmermann (1936), the typical form occurs in southern and central Europe, the western Mediterranean and north-west Africa and the subspecies *orientalis* Zimmermann in the eastern Mediterranean, north-east Africa, central Asia and across India, to as far as Indo-China. In India this species has often been erroneously called *E. dodecastigma* Wied.,* which is a quite distinct species and is

*Usually misspelled *dodecastigma* and wrongly credited to Mulsant (1850) ; Wiedemann's (1823) reference was omitted by Mulsant who later (1853) corrected this omission.

not related to *chrysomelina*. The larvae and adults of *E. chrysomelina* feed mostly on cucurbitaceous plants. In central Asia and India it is a pest, whereas in southern Europe it is not reported to do serious damage. The morphology of the larvae, although previously dealt with by Grandi (1913) and Klemm (1930), is treated here in greater detail and for all the instars. The examples of the larvae described here are from Eritrea, Palestine and India and belong to the subspecies *orientalis*. Some of the Eritrean specimens have rather dark coloration which is very variable.

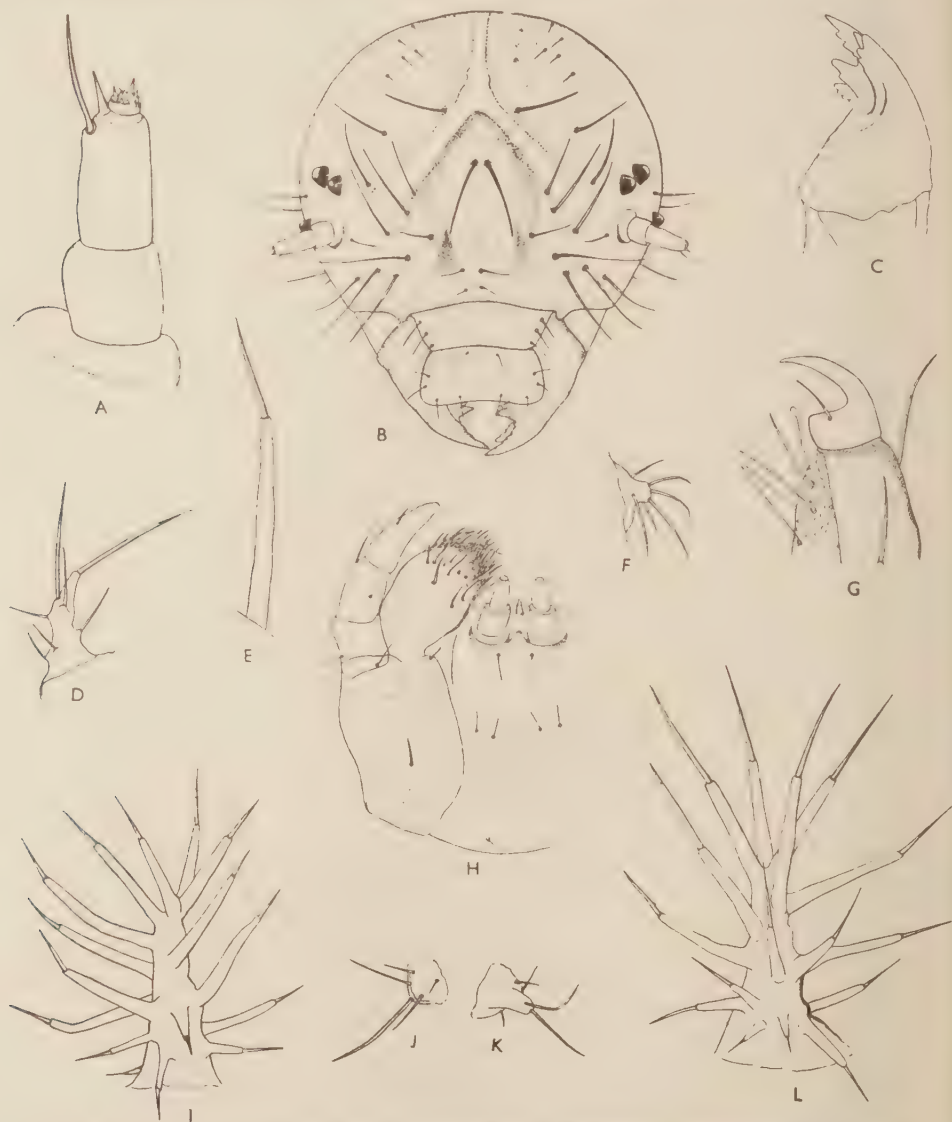


Fig. 6.—Larva of *Epilachna chrysomelina* subsp. *orientalis*: (a) antenna; (b) head; (c) mandible; (d) dorsolateral scoli of fifth abdominal segment; (e) subdorsal scoli of pronotum; (f) dorsolateral scoli of eighth abdominal segment; (g) claw; (h) maxilla and labium; (i) dorsal scoli of first abdominal segment; (j) ventrolateral struma of eighth abdominal segment; (k) the same of second abdominal segment; (l) dorsal scoli of pronotum.

Body elliptical, twice as long as wide, slightly narrower posteriorly than towards the head; a well grown, final-instar larva is 8 mm. long and 4 mm. wide across the third abdominal segment which is the widest. Colour usually light yellow, brownish in more heavily sclerotised parts; sometimes with dark brown pigmentation, especially at the apices of the scoli.

Head (fig. 6 *b*) subrounded, slightly longer than wide, brownish except for the epicranial suture and median part of the front which are lighter; clypeal suture darker; setae mostly long, a few short ones usually at the vertex and near the clypeal margin as shown in the figure; lower surface of the genae with three or four short setae. Ocelli three on either side, black, of equal size, and arranged triangularly; the two towards the vertex close together and situated a little distance away from the third which lies very close to and outside the antennal socket. Antennae (fig. 6 *a*) fairly long; the first segment nearly as wide as long, the second a little narrower than the first and only slightly narrowing anteriorly, nearly twice as long as wide, with a long seta and an elongate, conical sensilla at the apical margin; the third segment small, narrower, much shorter than wide and with a number of sensillae at the broad apex. Clypeus trapezoidal, narrow anteriorly, less than half as long as wide and with three or four moderately long setae at each lateral margin. Labrum nearly twice as wide as long, slightly rounded on the margins and with about six or eight moderately long setae; mandibles (fig. 6 *c*) much narrowed distally, each with four large teeth which are denticulate on the inner margin; maxilla (fig. 6 *h*) with the galea broadly oval, the distal part especially on the oral surface with fairly dense, rather delicate and moderately long setae; maxillary palpus with the apical segment long and narrowed distally; labium for the most part membranous, the small area round the bases of palpi sclerotised; the palpi with the apical segment narrower but a little longer than the basal one.

Thorax distinctly increasing in diameter towards the abdomen. Prothorax nearly twice as wide as long, with the pronotum oval, slightly more narrowed posteriorly, sclerotised with four or five shallowly pitted and darker areas situated near and posterior to the bases of scoli. Dorsal scoli (fig. 6 *l*) directed forwards and slightly upwards, its main stem elongate and conical, a little shorter than the length of pronotum with about 16 to 18 moderately long branches arising from all round its surface, and each with an apical brownish seta of varying length, the setae on the apical branches equal to or about two-thirds the length of the branches bearing them, those on the branches near the base proportionately small. Subdorsal scoli (fig. 6 *e*) consists of a single narrow process, which is a little shorter than the main stem of the dorsal scoli, and has the apical seta equal to about half the length of the process itself. Dorsolateral scoli similar to the dorsal scoli in size and structure but directed anterolaterally and a little dorsally. In addition, the pronotum has a pair of chalazae in the middle and about a dozen short chalazae at the posterior margin. Mesothorax shorter but distinctly wider than prothorax; dorsal scoli on each side closer to the subdorsal of the same side than to the middle of the segment, similar to the corresponding scoli on pronotum, directed forward and upward but with the branches reduced to about 12 and with shorter setae, each of the latter, half to one third as long as the branch bearing it; subdorsal scoli similar to the dorsal one with slightly longer setae; directed anterolaterally; the bases of dorsal and subdorsal scoli of the same side surrounded by a common, suboval, sclerotised and rather brownish area. Dorsolateral scoli similar to the subdorsal scoli but slightly narrower at base. Metathorax slightly greater in diameter than the mesothorax to which it is otherwise similar. Prosternum with a median struma consisting of about six moderately long setae; mesosternum and metasternum each with a pair of strumae, each struma situated close to and on either side of the median line and consisting of one long, central seta and five or six shorter ones. Legs with trochanter and femur together equal in length to the tibia; claw (fig. 6 *g*) with a short quadrangular basal tooth and with the distal part moderately bent near the base.

Abdomen : dorsal scoli (fig. 6 *i*) closer to the longitudinal median line than the corresponding scoli on the thorax ; the pair on any one segment with a common, transverse oval, sclerotised area round their bases ; on the first segment, vertical, each with 12 moderately long to long branches similar to those in the corresponding scolus on metathorax, on the second segment similar but directed slightly laterally, on the third to seventh segments, similar but directed gradually more posteriorly, and on the eighth, a little shorter with eight short branches bearing relatively longer setae. Subdorsal scoli situated in line with the corresponding ones on the metathorax, similar in structure to the dorsal scoli of the same segment, directed slightly more laterally and each with a sclerotised and sometimes pigmented area round its base, this basal area with a small notch on the side towards the middle of the segment. Dorsolateral scoli on the first segment directed laterally, slightly longer but narrower at the base than subdorsal scoli, each with usually 15 branches ; on the second to sixth segments gradually decreasing in length and in number of branches and directed more posteriorly in each succeeding segment, on the seventh segment reduced to nearly half the length of that on the preceding segment and with about eight setae arising from short tubercles ; on the eighth segment further reduced to a short process (fig. 6 *f*) with about six setae. Setae of dorsolateral scoli on the fifth (fig. 6 *d*) to eighth segments distinctly longer than those of the other scoli. Tergum of the ninth segment semicircular with about 12 short and six long setae, mostly on the external margin. The underside mostly with distinct, sclerotised and usually lightly pigmented strumae. Ventral strumae on the first segment each consisting of three short setae, one of which is longer than the other two, on the second segment consisting of four or five setae, two of which are longer, and on each segment from the third to sixth consisting of eight or nine setae, about four of which are longer ; on the seventh and eighth segments the ventral and subventral strumae of the same side are confluent, a character by which the species can be easily distinguished from others ; the total number of setae in these confluent strumae being usually ten and six in the seventh and eighth segments, respectively. Subventral struma absent on the first and often also on the second and when present on the latter segment it consists of only two short setae ; on the third segment the subventral struma bears four setae one of which is longer than the rest ; on each segment from the fourth to sixth, it consists of three long and six shorter setae. Ventrolateral struma on the first segment consists of a long seta borne on a short tubercle and a short seta close to it, on the second segment it consists of about seven setae, two of which are long and are borne on a short tubercle (fig. 6 *k*), on the third it is similar to that on the second but with the two tubercles sometimes confluent at the base and with the setae usually longer. On the fourth to sixth, its base is more convex and it has about seven setae, on the seventh and eighth it is shorter and has two long and four short setae (fig. 6 *j*). Ninth segment bears a total of 10 moderately long setae, mostly in the median part of the posterior margin, and the tenth has only a few very short setae along the transverse median lines.

The characters of the earlier instars are as follows :—

First instar : 0.8–1.25 mm. long and about 0.4–.75 mm. wide in the middle. Head with well defined epicranial and clypeal sutures, setae relatively long, ocelli as in the final instar, the antennae also similar but with the second segment only a little longer than its diameter and with a large sensilla, mouth-parts in general similar to those of the final instar except for the mandibles which have only the apical teeth serrate on the inner margin. Thorax slightly increasing in diameter posteriorly ; prothorax with the dorsal and dorsolateral scoli shorter than the length of the pronotum ; each with eight short branches bearing setae, nearly twice as long, at their apices ; the subdorsal scolus shorter, with the apical seta equal to twice its length. The scoli on the other thoracic segments and on the abdomen, longer, usually narrower at base, and with shorter setae, than the dorsal scoli on the prothorax.

Second Instar : 3.5 mm. long and 1.7 mm. wide, with the structure of the head similar to that in the first-instar larva, the scoli on the thorax and abdomen similar to those in the first instar, proportionately larger, with the number of branches usually increased to ten and bearing relatively shorter setae.

Third Instar : 4.5 mm. long and 2.25 mm. wide ; closely resembling the final instar, but scoli usually with ten to twelve branches.

Material examined : All instars and many examples of each collected and reared on watermelons in Eritrea (*G. de Lotto*). Several larvae collected and reared by the author on pumpkins, melons and watermelon leaves in the Punjab and Delhi, 1935-39. Palestine 1 larva (*Dr. F. S. Bodenheimer*).

Remarks : Besides the general structure of the armature of the body-wall, this species is characterised by the confluence of the ventral and subventral strumae on the seventh and eighth segments and can thus be easily separated from *Epilachna argus* (Geoffr.), *E. hirta* (Thnb.) and *E. vigintioctopunctata* (F.).

***Epilachna hirta* (Thunberg) (Fig. 7).**

Epilachna hirta is widely distributed on the African continent. The present material is from Eritrea.

Body oblong oval, much narrowed posteriorly, 6.5 mm. long and 2.25 mm. wide in the middle, coloration very variable, usually the greater part of head, posterior half of pronotum, longitudinal median and marginal part of mesonotum and metanotum, area round bases of abdominal scoli and lateral part of underside of body dark brown.

Head subrounded ; vertex and basal half of front with large and irregular, piceous patches except for the very light epicranial suture ; area surrounding ocelli, the antennae, ocelli and maxillary palpi piceous to dark brown ; setae moderately long to long, arranged as in *E. chrysomelina* except that the very short ones are absent ; ocelli also as in the latter species. Antenna (fig. 7a) with first segment slightly wider than long, second slightly narrower, nearly twice as long as wide, with a long seta situated a little below and a conical sensilla at the apical margin, third segment very short, less than half as wide as second and with a number of sensillae at apex. Clypeus trapezoidal, narrower anteriorly, about one-third as long as wide and with three setae on each lateral margin. Mouth-parts very similar to those of *E. chrysomelina*, but mandibles (fig. 7c) much narrowed distally, each with five sharply pointed teeth, two of which are larger ; one of the latter and two shorter teeth usually denticulate on the inner margin.

Thorax gradually increasing in diameter posteriorly, moderately convex dorsally. Prothorax nearly twice as broad as long, pronotum oval, sclerotised, dark brown to piceous mostly along the posterior margin and along the basal half of median longitudinal line ; dorsal scoli as long as pronotum, with the main stem conical slightly darker than the branches which arise from all round its surface, the branches being about 20 in number and, with the exception of about six near the base, moderately long and each bearing an equally long or slightly longer apical seta. The subdorsal scoli (fig. 7b) usually about two-thirds as long as the main stem of the dorsal scoli, narrow, divided into three short branches in the distal half ; each branch with relatively longer seta, usually equal to that on a branch of the dorsal scoli. Dorsolateral scoli similar to the dorsal scoli but with the apical setae on some branches much longer. A pair of chalazae with piceous setae present in the middle of the pronotum which has, in addition, a dozen short setae near its posterior margin. Mesothorax with the dorsal scoli arising at a point equidistant from the subdorsal and the median longitudinal line, directed upward, slightly longer but narrower at base than the dorsal scoli on the pronotum, with about 16 branches, similar to those on the latter scoli but with relatively shorter and stouter setae. Subdorsal scoli

similar but slightly longer, directed dorsolaterally and slightly anteriorly. Surrounding the bases of dorsal and subdorsal scoli of the same side is a subrectangular, dark brown to piceous area, with three triangularly placed darker and depressed spots in the middle. Dorsolateral scoli (fig. 7 *f*) similar to the subdorsal, directed anterolaterally and usually of lighter colour except near the base. Metathorax similar to mesothorax in arrangement and structure of the scoli. Underside with the area round the base of setae piceous; prosternum with a pair of moderately long setae in the middle; meso- and metasternum each with a pair of short strumae placed close to the median longitudinal line, each struma usually with four setae. Legs much darker on the outside; the femora nearly as long as the tibiae; claws (fig. 7 *d*) with the basal tooth subquadrate but rounded on the inner margin, the narrow distal part bent near the basal tooth.

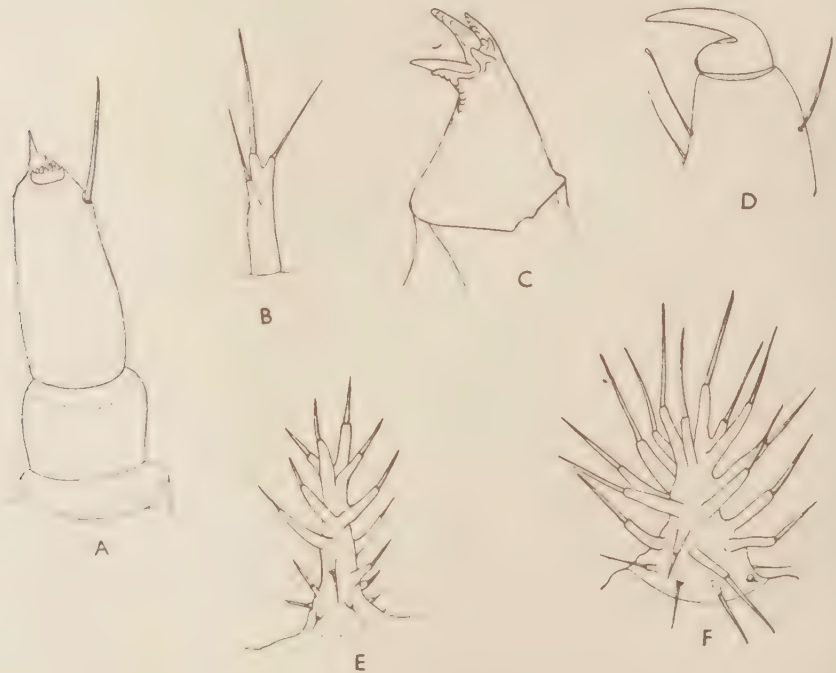


Fig. 7.—Larva of *Epilachna hirta*: (a) antenna; (b) subdorsal scoli of pronotum; (c) mandible; (d) claw; (e) dorsal scoli of second abdominal segment; (f) dorsolateral scoli of pronotum.

Abdomen with the first three segments only slightly wider than metathorax, the succeeding ones gradually narrowing; dorsal scoli of each segment placed closer to each other than are the corresponding ones on the meso- and metathorax, similar in structure to the latter on the first six segments (fig. 7 *e*), but with short and very short branches on the seventh and eighth segments, respectively; subdorsal scoli placed in line with the corresponding scoli on the last two thoracic segments and equal to them in length on the first six segments but with fewer (10 or 12) branches, the latter a little shorter on the seventh and eighth segments and with relatively longer setae; dorsolateral scoli on the first three segments similar to the corresponding ones on the meso- and metathorax but decreasing gradually in length and in number of branches from the fourth to the eighth segments, on the latter, present in the form of a conical projection, nearly as wide as long and bearing about six setae.

Tergum of the ninth segment semicircular, sclerotised and partially dark brown with about twelve moderately long setae; the tenth segment with a lightly pigmented area on either side, and bearing four very short setae. Underside mostly with the strumae sclerotised and pigmented; ventral strumae on first segment each consisting of a single short seta, on the second to seventh segment of three or four moderately long setae, on the eighth similar to that on the seventh but closer to the subventral struma of the same side; subventral strumae absent on first segment, represented by a single long seta on second, by one long and two short setae on third, by two long and four short setae on fourth to sixth and by one long and stout and two short setae on the seventh and eighth segments; ventrolateral strumae represented by two short setae on first segment, bearing three to six setae and a well developed chalaza in the centre on the second to fifth segments, similar on the sixth to eighth segments but without the chalaza. Ninth segment with a transverse sclerotised and pigmented area with three long and three short setae on each side. No setae present on the ventral surface of the tenth segment.

Material examined: Numerous full grown larvae (and other immature stages and adults) found feeding on *Solanum marginatum* at Asmara (cir. 2350 ft.)—ix.1947, in Eritrea (G. de Lotto).

***Epilachna vigintioctopunctata* (F.) (Figs. 8-10).**

Epilachna vigintioctopunctata is widely distributed in south-east Asia and Australia and occurs as a pest of solanaceous crops, such as potatoes and brinjals

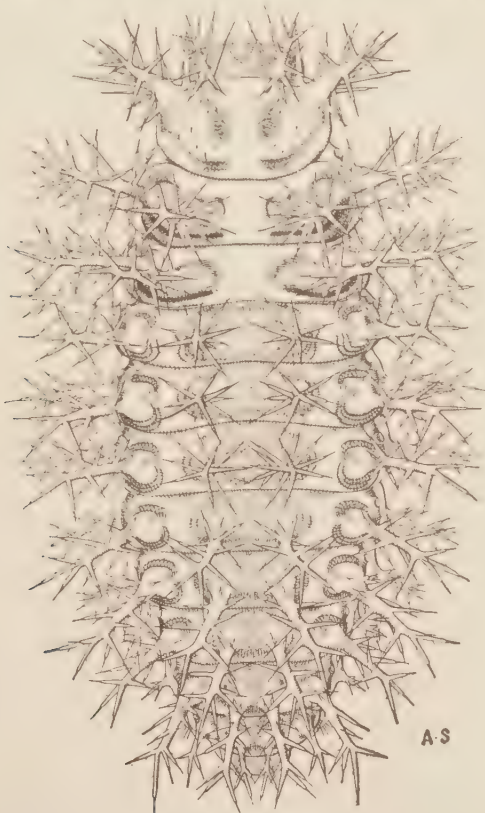


Fig. 8.—Larva of *Epilachna vigintioctopunctata*.

(egg-plant), in India, Ceylon, Malaya, East Indies and China. As in the case of several other species, the descriptions and figures of the larvae given in the works dealing with its biology and control (Lefroy 1909, Krishnamurti 1932 and Chue 1930) do not include details of structure by which it could be distinguished from related species. According to the classification based on the adults, this species is also divisible into several subspecies, but the examples of larvae examined from India (type locality) and Formosa do not show differences in structure except that some of those from Formosa have darker coloration.

Body (fig. 8) elongate oval, a little longer than twice its width; final instar larva about 6 mm. long and 2.8 mm. wide across the third abdominal segment; general colour pale yellow except for the more heavily sclerotised parts and areas round bases of scoli which are brown.

Head (fig. 9 d) subrounded; vertex, genae and greater part of front brown except for the ocelli and a small area round them which is dark brown; epicranial suture light in colour; setae usually moderately long, sparse and arranged as shown in the figure; ocelli three on either side, of equal size, arranged triangularly, the two towards the vertex situated close together and the third close to the base of the antenna; antenna (fig. 9 c) with the first segment nearly as long as wide, slightly narrower at base, second a little narrower, less than twice as long as wide, with a long seta and a sensilla situated a little below and at the apical margin, respectively, third segment small, nearly half as wide as second, about half as long as its own width, and with one long and a few short sensillae at apex; clypeus trapezoidal, narrow anteriorly, slightly dark towards base and with two short setae on each lateral margin; labrum nearly twice as wide as long, the upper surface with six or eight setae, the oral surface (fig. 9 f) with a row of short setae in the middle; mandibles (fig. 9 i) much narrowed distally, with five teeth, the two apical ones large and denticulate on the inner margins, the remaining three smaller and only bluntly denticulate; maxillae and labium as in *E. chrysomelina*.

Thorax slightly increasing in width towards the abdomen. Prothorax a little less than twice as wide as long, pronotum oval, sclerotised and light brown on each side of the median longitudinal line and bearing three or four simple setae at the posterior margin; dorsal scolus slightly longer than tergum, directed anterodorsally, fairly wide towards base, with 14 to 18 branches arising from all round its surface, the branches moderately long, except a few shorter ones situated close to the base, each bearing at the apex a brownish, stout, short seta (about half as long as a long branch) and two or three very thin, short, colourless setae which are usually dispersed irregularly in the median part; subdorsal scolus (fig. 10 e) like a large branch of the dorsal scolus, nearly as long as the stem of the latter, with the apical seta equal to three-fifths the length of the scolus, and with about five very thin colourless setae like those present on the branches of the dorsal scoli; dorsolateral scolus (fig. 10 f) a little long and broader at the base than the dorsal scolus, pointed anterolaterally and slightly upwards, with about 20 to 22 branches mostly as in the dorsal scolus, but some, especially those directed laterally, much longer and with longer but thinner setae. Mesothorax a little shorter than the prothorax, with the dorsal and dorsolateral scoli of the same side arising close together and surrounded by an oval, sclerotised, brown area, the dorsal scolus directed upward, slightly anteriorly and toward the middle of the segment and similar to the dorsal scolus on prothorax; subdorsal scolus slightly longer, directed dorsolaterally, but otherwise similar to the dorsal scolus. The dorsolateral scolus also similar to the subdorsal scolus but directed anterolaterally and without any sclerotised and pigmented area round its base. Metathorax slightly wider than but otherwise similar to the mesothorax. On the ventral side of the thorax the setae form strumae with moderately chitinised bases; prosternum with a single struma in the middle, usually consisting of five short setae, meso- and metasternum each with a pair of similar strumae close to the median

longitudinal line. Legs with the femora as long as the tibiae which are darker towards the apex; claw (fig. 9 *k*) with a subquadrate basal tooth and with the distal part moderately curved.

Abdomen: dorsal scoli on first seven segments similar to corresponding ones on metathorax but each with ten or twelve branches and with a shallowly pitted and dark spot on the outer side of the base; dorsal scolus on eighth segment equal to about two-thirds the length of the corresponding one on the preceding segments, narrower and with about eight short branches bearing rather long, thin and



Fig. 9.—Larva of *Epilachna vigintioctopunctata*: Antenna (*a*) first instar; (*b*) second instar; (*c*) fourth instar; Head (*d*) fourth instar; (*e*) first instar; (*g*) third instar; (*f*) underside of labrum of fourth instar; Mandible (*h*) first instar; (*i*) fourth instar; Claw (*j*) first instar; (*k*) fourth instar.

colourless setae ; subdorsal scoli (fig. 10 *l*) on first seven segments similar to dorsal ones but each with subrounded, sclerotised and lightly pigmented area round its base ; subdorsal scoli on eighth segment equal to about two-thirds the length of the dorsal one of the same segment but otherwise similar ; dorsolateral scoli also with distinct sclerotised area round their bases, similar in structure to the subdorsal scoli, or slightly longer on the first three segments, but decreasing in length in each successive segment, on the fourth to sixth segments with the branches gradually becoming shorter and fewer, being eight to ten in each scoli, but with the setae relatively long, thin and colourless, on the seventh segment the number of setae



Fig. 10.—Larva of *Epilachna vigintioctopunctata* : Subdorsal scoli (*a*), (*c*) first instar ; (*b*) second instar ; (*d*) third instar ; (*e*) fourth instar ; Dorsolateral scoli of pronotum (*f*) fourth instar ; (*g*) first instar ; (*h*) dorsal scoli of pronotum of first instar ; (*i* to *l*) subdorsal scoli of second abdominal segment of first, second, third and fourth instars, respectively.

reduced to about six, three of which are on short tubercles and one on a moderately long branch ; on the eighth segment the dorsolateral scolus reduced to a mere struma with about six setae, three of which are borne on short tubercles. Ninth tergite semicircular, sclerotised, pigmented light brown and with a dozen, rather long, thin and colourless setae situated mostly along the posterior margin. Tenth tergite membranous, except for a small, weakly sclerotised area on either side, and bearing two or three short setae. Ventral surface with chalazae or strumae which are neither sclerotised nor pigmented ; ventral and subventral strumae distinct on the first seven segments but contiguous on the eighth ; ventral strumae on first and second segments each consist of one long and three short setae, on the third to sixth segments the number of setae increases to six or seven, of which three are long and three or four short, and on the seventh and eighth segments the setae are reduced to four in each struma. Subventral struma absent on first segment ; on the second consisting of two setae and on the next six segments being similar to the ventral strumae. Ventrolateral group on the first segment consisting of a single chalaza, and on each of the succeeding segments consisting of a chalaza in addition to a number of short to moderately long setae, the number of setae being two and three on the second and third segments and varying from four to six on the next five. On the ninth segment the setae moderately long, not formed into groups, usually ten in number and arranged in a single row near the posterior margin. The tenth segment mostly membranous, with eight very short setae.

The characteristics of the earlier instars are as follows :

The larvae differ from those of the final or fourth instar in having lighter coloration and simpler armature.

First instar : 0.6 mm. long when newly emerged, 1.8 mm. long just before the first moult in the Punjab (which usually occurs four to six days after hatching) and about one-half to one-third as wide as long. Head (fig. 9 c) relatively large, like that of the final-instar larvae in general shape, and in the epicranial suture and arrangement of setae and ocelli, but with the antennae (fig. 9 a) slightly different in that the second segment is only a little longer than its diameter and has two sensillae at its apical margin. Mandibles (fig. 9 b) also differ from those of the final-instar larva, having three large and two very small and inconspicuous teeth, the apical one of the larger teeth being weakly denticulate on the inner margin. Thorax similar in arrangement of scoli to that of the final-instar larva ; the dorsal scolus (fig. 10 h) on pronotum with about eight short branches bearing slender setae usually much longer than the branches, subdorsal (fig. 10 a) usually comprising a single filiform projection bearing a long seta, though sometimes one of the pair is (fig. 10 c) divided in the middle into two branches, each bearing a seta ; dorsolateral (fig. 10 g) similar to dorsal but slightly larger and with about twelve branches. Scoli on the other thoracic segments similar in general to the dorsolateral scoli on pronotum. On the underside each segment with a pair of ventral setae, each situated close to and on either side of the median longitudinal line. Legs relatively long, with the basal tooth of the claw (fig. 9 j) rather small and triangular, unlike the large, quadrangular one of the final instar. Abdomen with short scoli bearing relatively long setae, each scolus (fig. 10 i) on the first few segments having one long apical branch and three or four short branches. On the ventral surface, the ventral, subventral and ventrolateral groups represented by a single seta each.

Second Instar : 3 mm. long, 1 mm. wide across the middle of the body. Similar to first instar in general structure and antennae (fig. 9 b) but easily distinguished from it by the structure of the scoli, the dorsal and dorsolateral scoli on thorax having nine branches and on abdomen seven or eight (fig. 10 j) ; further, the small inconspicuous setae that are present on the branches in the final instar first appear in the second instar. On the ventral surface each group comprises three or four setae. Duration usually shorter than that of the first instar, being only two or three days in the Punjab.

Third Instar. : 5 mm. long and 2.25 mm. wide. Similar to fourth instar in general structure. Head : (fig. 9 g). Pronotum and parts of the thoracic tergites more conspicuously sclerotised than in the earlier instars. Thoracic scoli usually with about twelve relatively long branches, abdominal scoli (fig. 10 k) on the first few segments each with about twelve branches with relatively short setae ; undersurface with the arrangement and number of setae almost the same as in the fourth instar. The duration of the third instar varies from three to six days.

Material examined : More than 12 larvae of each instar (and reared pupae and adults) from Formosa, 1937 (T. Yoshida) and several larvae in each instar from the Punjab and Delhi (India) reared by the author.

***Epilachna dentulata* Dieke (Plate VI, c, d).**

Epilachna dentulata is very closely related to *Epilachna vigintioctopunctata*. The adults of both species are similar in general appearance and in elytral spots. The larvae of these two species also agree in many structural details, but show greater differences in general appearance than the adults.

Body comparatively dark brown on the sclerotised parts and at the apices of the branches of scoli, on the underside the bases of strumae dark brown.

Head similar to *E. vigintioctopunctata* in shape, colour, arrangement of setae and ocelli, and structure of antennae and mouthparts.

Thorax differs from that of *E. vigintioctopunctata* mostly in the structure of the scoli which are longer and have more sparsely placed branches, the individual branches long but the apical setae short, as in *E. vigintioctopunctata*. Pronotum with the dorsal scoli each having about 17 branches of which six, placed near the base, are short, the subdorsal scoli nearly four-fifths the length of the main stem of the dorsal one and bearing a long apical seta as in *E. vigintioctopunctata* ; the dorsolateral scoli similar to the dorsal in length and number of branches, but slightly broader at base. Mesothorax and metathorax similar as regards the scoli, which are slightly longer than the corresponding ones on the pronotum, each scoli with about 15 branches of which four, situated near the base, are short while the others are long, the apical setae dark-brown, stout, and each equal to one-fourth the length of the branch bearing it. Underside with a pair of strumae on prosternum instead of a single one as in *E. vigintioctopunctata*, each with one long and usually two short setae ; mesosternum and metasternum each with a pair of larger strumae with brown coloration, each struma with a centrally placed long seta and four or five shorter ones around it.

Abdomen similar in general shape and arrangement of scoli to that of *E. vigintioctopunctata* ; the scoli with the main stem and branches relatively long, the latter sparse, each with a short, brown apical seta as in the thoracic scoli. On the underside the strumae more transverse on first three segments and each comprising three rather short setae, those on fourth and fifth similar but each with four short setae, those on sixth to eighth segments rather sub-rounded and each with three moderately long setae ; on eighth segment ventral and subventral strumae close to each other but not contiguous as in *E. vigintioctopunctata*. Subventral struma absent on first segment, represented by a single seta on the second, by four to six, short to moderately long, setae on third to sixth segments, and by three and two setae on seventh and eighth segments, respectively. Ventrolateral group consists of a single short seta on first segment, of one short seta and one long chalaza on second, and third segments, of one long chalaza and three or four shorter setae on fourth to eighth segments. Ninth segment with the greater part of sternum sclerotised, brown and with a total of about twelve moderately long setae situated along the posterior margin ; tenth segment without setae.

Material examined: 16 larvae, some feeding on *Solanum xanthocarpum* and *Solanum* sp., at Dehra Dun, United Provinces, India, collected and some reared to adults by J. C. M. Gardner (July, 1928 : 1933), Balwant Singh (August, 1933) and A. K. Sharma (June, 1941).

Epilachna flavofasciata (Laporte) (Pl. VI a, b, text fig. 11).

Epilachna flavofasciata is widely distributed in South America from Colombia to Bolivia. The larvae were briefly described by Candèze (1861, as *proteus* Guér.), who regarded it as very similar to the European species *E. argus*.

Body (Pl. VI a, b) elongate oval, widest in the middle, more convex on the upper than on the ventral surface; final-instar larva usually 10 mm. long and 3.5 mm. wide in the middle. Upper surface piceous, with a light streak on the thorax along the median longitudinal line and scoli whitish except for the piceous setae at the apices of the branches; underside lighter in colour, with small irregular, lightly piceous patches, near bases of setae; legs mostly dark brown.



Fig. 11.—Larva of *Epilachna flavofasciata*: (a) antenna; (b) mandible; (c) claw; (d) subdorsal scolus of pronotum; (e) dorsolateral scolus of pronotum.

Head dark brown to piceous with the epicranial and frontoclypeal sutures, the lateral part external to the ocelli, the clypeus and labrum paler; setae brownish, short to fairly long, vertex with six, short setae dispersed sparsely on either side; a pair of long setae in the centre of the front, other setae on the front arranged as in *E. borealis*; on the underside of the genae on either side usually six shorter setae. Ocelli three on either side, the two towards the vertex very close together, third situated close

to the antennal socket ; antenna (fig. 11 *a*) dark brown, first segment slightly wider than long, second slightly narrower than first but nearly twice as long as wide, with a long seta near apex, third brownish, very small, much narrower than second, nearly one-third as long as wide, bearing a few sensillae at apex ; clypeus trapezoidal, narrow and membranous anteriorly ; labrum slightly narrower at base, and twice as broad as long ; clypeus and labrum each bearing usually four setae on each lateral margin ; mandibles (fig. 11 *b*) dark brown, distal half narrow with five large teeth, the apical two of which are bluntly denticulate on the inner margin, the subapical teeth sharply pointed and not denticulate ; galea oblong, a little narrowed distally, sclerotised near the base and with long, dense setae ; maxillary palpi long, the apical segment narrow and equal to the total length of the two preceding segments.

Thorax gradually increasing in width towards the abdomen. Prothorax transverse, nearly one-and-a-half times as wide as long, its tergum oval, slightly more rounded on posterior margin, dark brown to piceous except, as mentioned above, a narrow paler longitudinal streak in the middle and the whitish scoli. The dorsal scolus as long as tergum, moderately broad at base and gradually tapering, with eleven moderately long and four short branches, each bearing a stout, piceous seta, usually equal to two-thirds the length of the branch itself but, sometimes, especially on the apical branches twice as long as the branch itself and thin and lighter in colour, the branches near the base arising from all round the main stem of the scolus but tending to be arranged rather bilaterally towards the apex ; subdorsal (fig. 11 *d*) scolus about one-third as long as dorsal and narrower, the main stem usually with a long, stout and piceous seta at apex and three or four short branches situated in the basal two-thirds, each bearing a dark seta as long as itself ; dorsolateral scolus (fig. 11 *e*) longer than dorsal arising from anterolateral margin of tergum and pointing anterolaterally and slightly upwards, with ten moderately long and ten rather short branches similar to those on the dorsal scolus ; the rest of the tergum with many short and piceous setae near posterior margin and a pair of chazae with whitish base on either side. Mesothorax shorter than prothorax ; dorsal scolus similar to that on the latter, pointing in the same direction but short and with fewer, usually eight, branches each bearing a seta as long as itself ; subdorsal scolus longer than dorsal, with 12 to 14 branches, those in the median part tending to be arranged bilaterally, the setae much shorter than the branches ; dorsolateral scolus shorter than subdorsal but with the same number of branches. Metathorax similar to mesothorax in general shape and in arrangement and structure of scoli. On the underside, prosternum with a pair of dark ventral strumae, each consisting of six to eight long, brown setae, the number of setae in a struma increasing to between ten and twelve on the mesosternum and metasternum. Legs with coxae on each segment well separated from each other, femur nearly as long as tibia, claw (fig. 11 *c*) with the basal tooth subtriangular and the distal part sharply bent near the tooth.

Abdomen : The pair of dorsal scoli on each segment surrounded at base by a common, large, oval, strongly sclerotised, piceous area ; those on the first four segments equal in length to the corresponding scoli on the metathorax, each with about nine rather short branches, arranged almost bilaterally and bearing slightly shorter, apical setae ; those on the fifth to seventh segments gradually increasing in length and in number of branches until, on the seventh, they are equal to about twice the length of those on the first and bearing about twelve, long to short branches (the shorter ones near the base), with shorter setae ; on the eighth the dorsal scoli about half the length of those on the seventh and with fewer branches. Subdorsal scoli much longer than dorsal ; on the first six segments with about twelve moderately long branches bearing short setae and arranged more sparsely and bilaterally, those on the seventh segment equal to nearly two-thirds the length of the corresponding ones on the sixth and with the branches rather close, those on the eighth equal to half the length of those on the seventh and nearly half as wide at the base, with short

tubercles bearing long setae. Dorsolateral scoli directed laterally except for those on the last three segments which are diverted slightly posteriorly; on the first four segments similar and equal in length to the subdorsal ones of the same segment but decreasing rapidly in length on each segment from the fifth to eighth, becoming merely a rounded slightly convex protuberance bearing about eight to ten moderately long setae. Ninth tergite semicircular, uniformly sclerotised and dark brown, bearing 20 to 30 moderately long setae situated near the external margin. Tenth segment short, mostly membranous except for a dark sclerotised patch bearing a few short setae on each side of the tergum. On the ventral surface, the setae arise from dark spots mostly scattered irregularly but some especially long setae, arising close together and forming small ill-defined strumae; ventral strumae consisting of two to four setae on the first eight segments, subventral absent on the first and consisting of one to three setae on each of the succeeding seven segments, ventrolateral almost alike on all the segments but each with one or sometimes two chalazae bearing very long setae. Ninth segment with about eight or ten long setae arising from a moderately sclerotised brownish area, tenth lightly pigmented with two or three short setae on either side.

Material examined: Over 50 larvae (and adults, collected together) feeding on leaves of *Datura* sp., from Colombia, 1916 (M.T. Dawe).

***Epilachna eusema* (Weise) (figs. 12 and 13).**

Epilachna eusema occurs in Tucumán, Argentina; the larva has not been described before.

Body (fig. 12) similar to that of *E. flavofasciata* in general shape but smaller, 6 mm. long, 2 mm. wide across the third abdominal segment. General colour light yellowish, except for a dark brown area between the dorsal abdominal scoli.

Head with the vertex (except the epicranial suture) and the proximal part of the front with small brown patches; epicranial suture with a short stem and rather indistinctly defined owing to the generally lighter colour of the head; setae light, moderately long, rather sparse, absent on the vertex, three pairs in the median area, and about sixteen setae on either side, arranged as in *E. flavofasciata*, ocelli three on either side; dark brown, of almost equal size, and placed triangularly, one near base of antenna, and two a little away towards vertex and close together, the outer one being slightly higher; antenna (fig. 13 *a*) three-segmented, with the first segment nearly as long as wide, the second narrower, slightly more so distally, nearly twice as long as wide, with a moderately long seta and a conical sensilla near the apical margin, third segment very small, ring-like, with a few small and one moderately long, sensillae at the apex; clypeus trapezoidal, four times as broad as long, much narrowed distally, the lateral margins each with three short setae; labrum (fig. 13 *d*) a little more than twice as broad as long, slightly rounded at the anterior angles and with a dozen short setae; mandibles (fig. 13 *b*) with three to four large and two short teeth in the distal part, the two large apical teeth denticulate on the inner margin; maxilla (fig. 13 *c*) with the galea elongate oval and bearing rather dense and long setae on its distal part, maxillary palpi long, with the apical segment as long as the two preceding segments together, narrowed distally; mentum with the palpi moderately long, the apical segment being about twice as long as wide.

Thorax (fig. 12) only slightly increasing in width towards the abdomen. Prothorax a little less than twice as wide as long, tergum subrounded laterally, sclerotised and having, in addition to the three pairs of scoli, two pairs of chalazae in the middle and a number of smaller chalazae on posterior margin; dorsal scolus one-and-a-half times the length of the tergum, situated a little below the anterior margin, directed upwards and forwards, and with about twelve short branches arranged more or less bilaterally, each branch bearing an apical seta which is slightly shorter than the

branch itself; subdorsal scolus (fig. 12) about half as long as dorsal, usually situated at anterior margin of tergum but in one example coalescent with the dorsolateral scolus, the main stem narrow, bearing a short apical seta and three or four short branches, each with an apical seta as long as itself; dorsolateral scolus longer and broader at the base than the dorsal, directed anterolaterally and a little upwards, with about 26 moderately long to short branches, the long ones about 16 in number arranged almost bilaterally in distal half of main stem, the remainder short, close together and arising from all round the proximal part of the main stem. Mesothorax a little shorter than prothorax but slightly wider; tergum rectangular; dorsal scoli as long as the tergum, similar to the corresponding ones on pronotum with usually ten short branches arranged bilaterally; subdorsal scolus situated at a fair distance from the dorsal and rather near the lateral margin of the tergum, directed anterolaterally and slightly upwards, nearly twice as long as the dorsal, narrowing anteriorly and having about 24 rather short to very short branches similar to those on the other scoli, and each bearing a seta as long as itself at its apex; dorsolateral scolus pointed in the same direction as the subdorsal, narrower at base than the latter and nearly two-thirds as long, with about 12 branches similar to those in the other scoli; there are also a few setae on short tubercles scattered irregularly on the tergum.

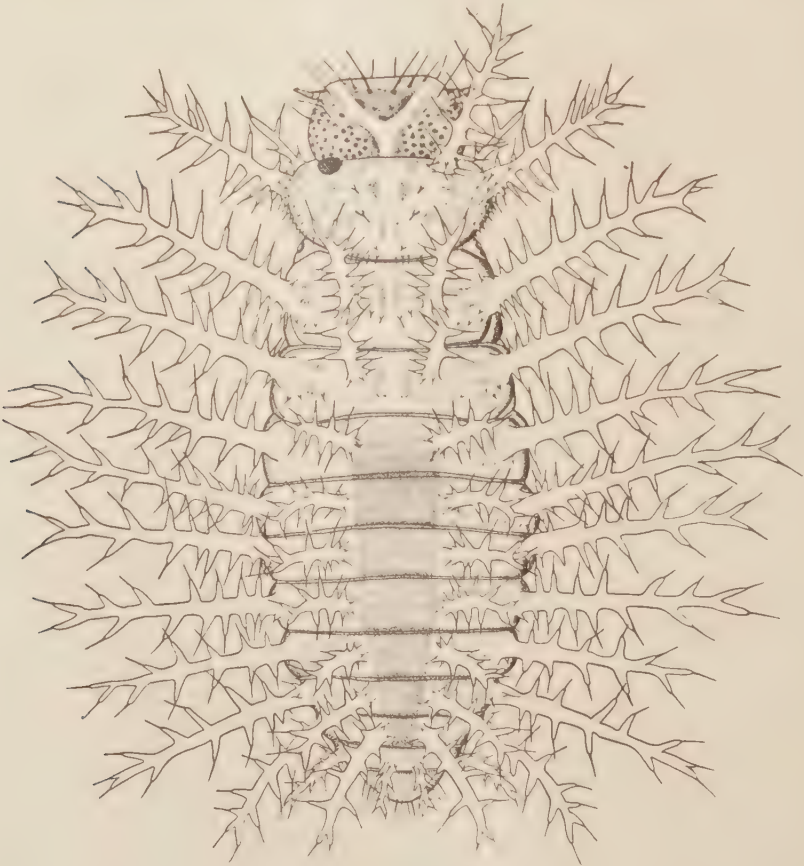


Fig. 12.—Larva of *Epilachna eusema* (left dorsal scolus on pronotum and dorsolateral scoli on other segments not shown).

Metathorax similar to mesothorax, slightly wider. On the underside, each segment with the ventral group represented by three short setae situated close to and on either side of the longitudinal median line. Legs with the coxae on the same segment fairly well separated, claw (fig. 13 c) with a subquadrate basal tooth, and with the distal part narrow, pointed, and sharply bent near the tooth.



Fig. 13.—Larva of *Epilachna eusema*: (a) antenna; (b) mandible; (c) claw; (d) labrum; (e) maxilla and part labium; (f) ventral half of fifth to seventh abdominal segment (dl=dorsolateral; sd=subdorsal).

Abdomen: scoli arranged almost in line with those of the metathorax; dorsal scoli on first segment equal to the corresponding ones on metathorax, directed laterally and a little anteriorly, each with about 12 short, bilaterally arranged branches bearing short apical setae; dorsal scoli on the succeeding six segments with the same number of branches as those on the first, their length gradually increasing on the succeeding six segments. On the eighth segment, they are reduced to two-thirds the length of those on the seventh and have only nine short branches; subdorsal scoli on first six segments similar to those on metathorax, equal to them in length and pointing in about the same direction, each with about 20 branches

bearing short, rather stout, dark setae at their apices ; on the seventh a little shorter ; on the eighth nearly half as long, with short branches which are, however, not arranged bilaterally ; dorsolateral scoli mostly directed laterally, on the first four segments equal to about two-thirds the length of the subdorsal scoli of the same segments, each with about 12 branches ; on the fifth to seventh (fig. 13 *f*, *dl*) segments the dorsolateral scoli markedly decreasing in size in each succeeding segment, while those of the eighth are merely short, conical processes, each with one long and three or four short setae. Ninth tergite semicircular, sclerotised, with about 18 long and 6 short setae situated mainly on the external margin. Tenth segment without setae. On the ventral surface the setae not forming strumae, rather short and sparse on the first three segments ; on the succeeding segments becoming longer and with the decrease in the size of the segments, becoming less sparse ; usually a pair of setae on either side of mid-ventral line longer than the rest (fig. 13 *f*). On the ninth segment about eight short to moderately long setae near the distal margin ; tenth segment without setae.

Material examined : 16 larvae of various instars from Tucumán, Argentina (*H. L. Parker*) ; on loan from U.S. National Museum, Washington.

Genera *SUBCOCCINELLA* Guérin-Ménéville and *CYNEGETIS* Chevrolat *in* Dejean.

According to the classification based on the adults, the genera *Subcoccinella* Guér. and *Cynegetis* Chevr. are closely allied. This is supported by the structure of the larvae, which are similar in general shape, and the armature of the body-wall. The two, however, show important differences in the structure of the head as given in the key to the genera and in the description of the larva of *Cynegetis impunctata* (L.). The strongly sclerotised triangular area of the front of cranium, the anteriorly produced lower part of genae, the structure of mouth-parts, especially of the mandibles, are modifications which seem to be related to special feeding habits. In fact, very similar structural modifications occur in *Chnootriba similis* (Thnb.) which feeds mostly on wheat, maize and other Gramineae but is otherwise not related to *Cynegetis*. Very little is known of the food-plants of the latter but wheat (*Triticum repens*) is one of the plants on which it is reported to feed. It is probable that further observations may prove that it feeds mainly on Gramineae or that its food-plants are at least different from those of *Subcoccinella*.

***Subcoccinella vigintiquatuorpunctata* (L.) (Figs. 14 & 15).**

Subcoccinella vigintiquatuorpunctata is the only phytophagous species of lady-birds occurring in the British Isles. It is distributed all over Europe and the neighbouring countries of Asia and North Africa. The larvae and adults feed mostly on clovers, lucerne, vetches, etc., which, however, are not damaged to any great extent. From a taxonomic point of view, the species is of interest, being the genotype of *Subcoccinella* and resembling fairly closely another European, monotypic genus, *Cynegetis*, from which it may be distinguished by the characters given in the key. Huber (1842) described morphological details of the larvae. Strouhal (1927) described the mandibles and certain scoli. Marriner (1927) gave an account of the life-history in Britain.

Body (fig. 14) elongate oval, with the greatest width at the third abdominal segment, slightly narrowing anteriorly, much more so posteriorly, a fully grown larva of the last instar is about 4.4 mm. long and 2 mm. wide. General colour yellowish with certain, very variable, light to dark brown markings on head and tergites ; in the first instar these markings are entirely absent.

Head (fig. 15 *a, b*) subrounded, slightly broader than long, vertex and proximal part of the front with small dark brown patches which are usually very variable ; epicranial suture consisting of lightly coloured coronal and frontal sutures ; the fronto-clypeal margin uniformly light brown and very narrowly produced backwards for a short distance along the median longitudinal line ; setae arranged as shown in the figures, usually long except in the median anterior part of the front which has about six pairs of short setae ; ocelli three on either side, arranged triangularly, the two towards the vertex not very close to each other, though the distance between them is less than their distance from the third which is situated close to the antennal socket ; the median ocellus, situated more towards the centre of the head, slightly larger than the other two ; antennae (fig. 15 *f, g*) two-segmented, the first segment

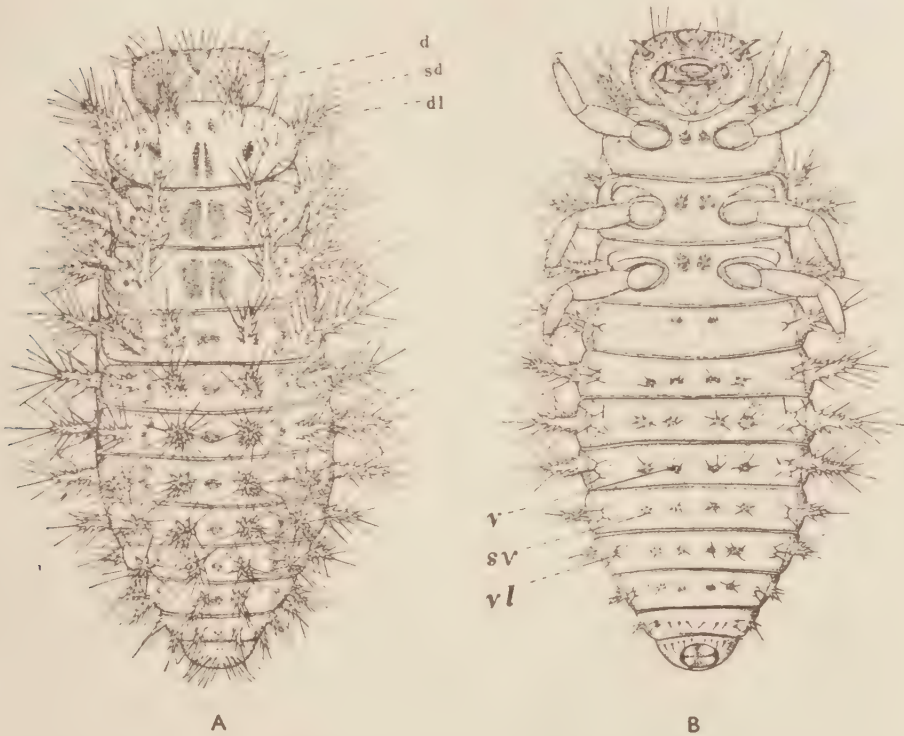


Fig. 14.—Larva of *Subcoccinella vigintiquatuorpunktata*: (a) dorsal view ; (b) ventral view (d=dorsal scolus ; sd=subdorsal scolus ; dl=dorsolateral scolus ; v=ventral struma ; sv=subventral struma ; vl=ventrolateral struma).

short, nearly half as long as wide, the second narrower, slightly more so distally, bearing a long seta at two-thirds and a number of sensillae, one of which is longer than the rest, at the apex (fig. 15 *g*) ; labrum nearly three times as wide as long ; mandibles (fig. 15 *h*) very broad at base, slightly narrowed distally, with two large apical teeth weakly denticulate on the inner margin and three subapical ones, two of which are moderately large and blunt, and the third small and conical ; maxilla (fig. 15 *a*) with an oblong galea bearing moderately long and dense setae distally ; palpus with the apical segment filiform, a little narrower but longer than the preceding segment.

Thorax only slightly increasing distally. Prothorax nearly twice as wide as long, pronotum oval, with variable markings and pitted areas; dorsal scoli (fig. 15 *d*) conical, a little shorter than length of pronotum, broad at base, nearly half as broad as long, with about 18 to 20 short branches each bearing a very long apical seta which is usually over four times the length of its branch, branches near base of scoli very short or like tubercles; subdorsal scoli (fig. 15 *c*) in the form of a moderately long, filiform process with an equally long seta at apex; dorsolateral scoli similar to the dorsal but situated at anterolateral angle of pronotum; in addition there are a number of simple setae, mostly along posterior margin of pronotum. Meso- and metathorax a little shorter than pronotum, each with the tergum having an elongate

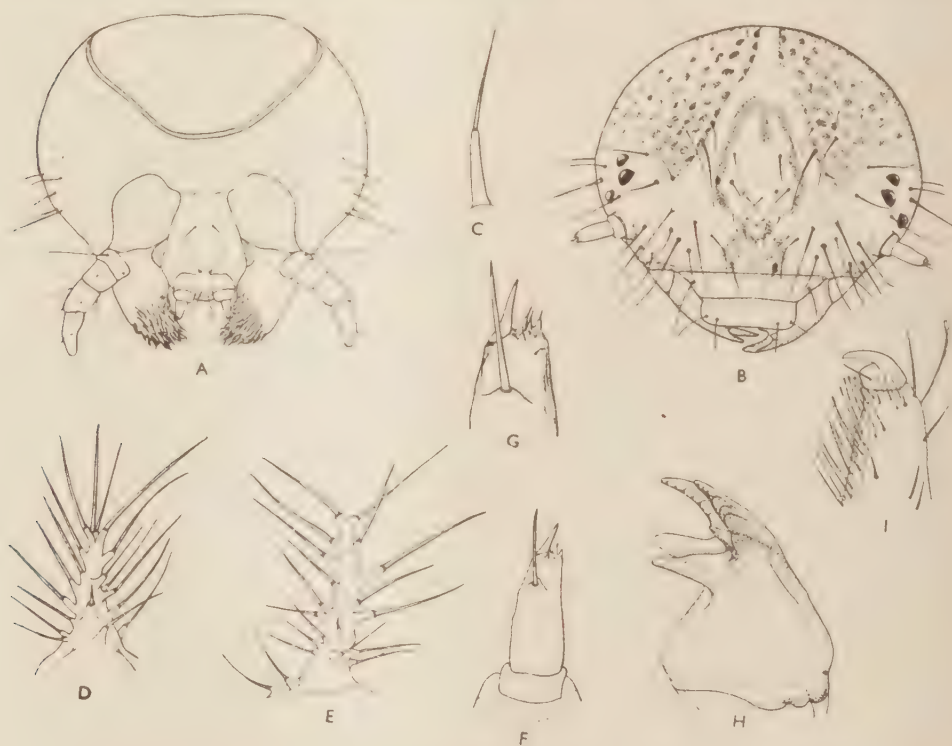


Fig. 15.—Larva of *Subcoccinella vigintiquatuorpunctata*: (*a*, *b*) ventral and dorsal view of head; (*c*) subdorsal scoli of pronotum; (*d*) dorsal scoli of pronotum; (*e*) subdorsal scoli of second abdominal segment; (*f*) antenna; (*g*) apex of antenna much enlarged; (*h*) mandible; (*i*) claw.

brown marking on either side of the median longitudinal line and two or three dark patches between the dorsal and subdorsal scoli on either side; dorsal scoli similar to the corresponding one on pronotum but narrower at base and situated a little farther from the median longitudinal line and almost in the centre of the half of the tergum, subdorsal and dorsolateral scoli similar to the dorsal one of the same segment, the dorsolateral directed anterolaterally; on the underside each segment with a pair of ventral strumae situated close together, each with four or five short setae; coxae of any one segment close together, claw (fig. 15 *i*) with a subquadrate basal tooth and moderately bent near it.

Abdomen: the first eight segments each with three pairs of scoli and with brown to dark brown, variable markings on the tergum, the markings comprising a pair

situated in the middle of the segment and others between the dorsal and subdorsal scoli; dorsal scoli on first five segments similar to metathorax but gradually decreasing in size, those on the sixth to eighth with fewer branches, those on the eighth having only six or seven, which are directed posteriorly; subdorsal scoli (fig. 15 *e*) similar to the dorsal of the same segment; dorsolateral scoli also similar on the first four segments but decreasing gradually in length and in number of branches and setae on the succeeding segments. Ninth segment with a semicircular, uniformly sclerotised tergum with a dozen moderately long setae situated mostly near the external margin. The ventral surface (fig. 14 *b*) with the strumae rather strongly sclerotised but not deeply pigmented; ventral strumae on first segment each consisting of two transversely-placed, short setae, on the second to sixth segments each struma with about four or five short to moderately long setae, on the seventh and eighth segments each with three setae; subventral strumae on first segment each represented by three or four short setae, on the other segments similar to the ventral strumae of the same segment; ventrolateral strumae on first segment each represented by a single chalaza, on the second by a conical projection bearing three setae, on the third to sixth segments similar to the other strumae of the same segment but usually with an additional chalaza. Ninth segment with about a dozen setae situated close to each other and to the posterior margin; tenth segment without setae.

Material examined: Many larvae from England and Wales in the British Museum (Nat. Hist.) collection and others collected and reared by the author; one larva from Denmark, Maglehoi Honkenya, 3.vii.1946 (J. P. Kryger).

***Cynegetis impunctata* (L.) (Fig. 16).**

Cynegetis impunctata occurs on the continent of Europe and in the western part of Russia. Its feeding habits are inadequately known, the recorded food-plants being wheat (*Triticum repens*), bilberry and *Trifolium*. Böving (1917), while enumerating the general characters of the EPILACHNINAE, pointed out a few structural peculiarities of this species differentiating it from three other species of the subfamily studied by him.

Body similar in outline and size to that of *Subcoccinella vigintiquatuor punctata* (L.), but the body, in the examples seen, though pale yellow, has only a few brown markings on the tergites.

Head (fig. 16 *c*) subrounded; vertex and median part of front with small, brown, irregular patches which are very rarely coalescent on the vertex but frequently so on the front; frontoclypeal margin strongly sclerotised, brown, and with the median part triangular (fig. 16 *c*, *t*) and pointed towards the centre of the head for a short distance; the lower part of the genae (fig. 16 *c*, *g*) distinctly produced forwards; setae moderately long, less numerous than in *vigintiquatuor punctata* and usually arranged as shown in fig. 16 *c*; ocelli differ in size and arrangement from those in the latter species, three on either side, conical, arranged triangularly, the two towards the vertex closer to each other and larger than the third situated near the antennal socket. Antennae (fig. 16 *a*) two-segmented, with basal segment short, twice as wide as long, the second a little over twice as long as wide and much narrowed towards the apex (fig. 16 *b*), with a long stout seta at two-thirds its length and with three or four long to short, conical, sensillae at the apex; clypeus trapezoidal, much narrowed distally; labrum small and subquadrangular; mandibles (fig. 16 *e*) elongate, much narrowed distally, with a large apical tooth, which is denticulate on the innerside, and three small, subapical teeth which are not denticulate; maxilla and labium rather similar to those of *Subcoccinella*.

Thorax similar to that in *S. vigintiquatuor punctata* in general shape and armature of the body-wall, except that the scoli are relatively shorter; pronotum with numerous setae dispersed irregularly, and with a few chalazae on the lateral margins. On the

ventral surface each segment with a pair of long setae, each situated on either side of the median ventral line. The group of setae and chalazae situated on the area external to the first pair of coxae was considered characteristic of the genus by Böving (1917), but seems to be only slightly larger than that present in *Subcoccinella*.

Abdomen also similar to that in *S. vigintiquatuorpunctata* in general shape and arrangement and structure of scoli, which are, however, relatively shorter. On ventral surface, strumae also similar in arrangement but very weakly sclerotised at base, not pigmented and with fewer setae. The ventral strumae on the first, eighth and



Fig. 16.—Larva of *Cynegetis impunctata*: (a) antenna; (b) apex of antenna much enlarged; (c) head (t, median part of frontoclypeal margin; g, lower part of geneae); (d) dorsolateral scolus of seventh abdominal segment; (e) mandible; (f) subdorsal scolus of pronotum; (g) claw; (h) ventrolateral struma of seventh abdominal segment; (i) same of eighth abdominal segment; (j) dorsolateral scolus of pronotum; (k) subdorsal scolus of second abdominal segment; (l) ventral view of the left half of sixth abdominal segment showing ventral (v), subventral (sv) and ventrolateral (vl) strumae.

ninth segments each represented by two long setae and on the second to seventh segments by three or four setae (fig. 16 l v), the subventral strumae absent on the first segment, and are represented by two or three setae (one of which is longer than the others) on the next seven segments (fig. 16 l, sv); ventrolateral strumae on the first eight segments (fig. 16 h, i) each consisting of two or three setae and about the same number of chalazae, one of which usually has much longer seta than the rest. The subventral strumae on the seventh and eighth segments with the base more convex than on the sixth. The ninth segment with twelve short setae near the posterior margin; the tenth entirely devoid of setae.

Material examined: 5 larvae (and a pupa and adult), Berlin, July, 1928 (R. Korschefsky), lent by Dr. F. van Emden.

Genus *AFISSA* Dieke.

Since the larvae of only a single species of this genus are known, the characters given in the key to the genera (page 165) are only provisional. The absence of the subdorsal scoli on the pronotum, however, may prove characteristic; they are also absent in the larvae of another species of this subfamily which were collected in the Kumaon Hills (c. 4,000 ft.), India, and may well belong to this genus, though they cannot be identified because of lack of adults. In details of the structure of the mandibles, which are very large, and of the armature of the body-wall, the two species are very distinct.

Afissa dumerili (Mulsant) (Figs. 17 and 18).

Originally described from "les Indes orientales" (under which Mulsant frequently included examples from India), this species is recorded by Dieke (1947) from North Bengal and Assam. Examples of it from the Nilgiri Hills (South India), Bombay, Bengal, Assam, Sikkim, the Andaman Islands, Burma and Siam are present in the British Museum (Nat. Hist.). The larvae described here are from North Bengal.

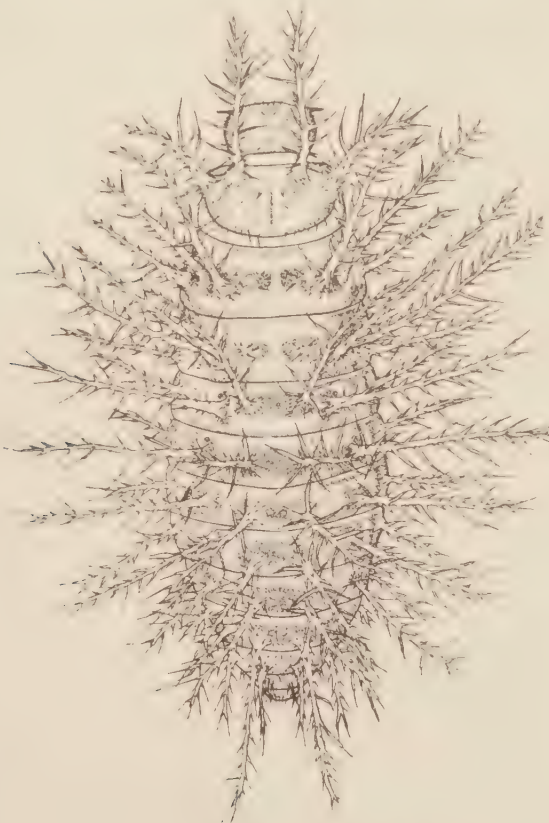


Fig. 17.—Larva of *Afissa dumerili*.

Body elongate oval, 7.5–8.0 mm. long and about 2.6 mm. wide in the middle ; last two thoracic segments only a little narrower than the first four abdominal ones ; normally light yellow with the more heavily sclerotised parts, such as the head, legs and the area round the bases of the scoli, light brown.

Head (fig. 18 *d*) rather small relative to size of body, brown, the coronal and frontal sutures lighter ; setae short to long, rather sparse, arranged as shown in the figure ; ocelli three on either side, of almost equal size arranged triangularly, the two towards the vertex placed obliquely and not very far from the third which is situated near the base of the antenna ; antenna (fig. 18 *a, b*) long and narrow,

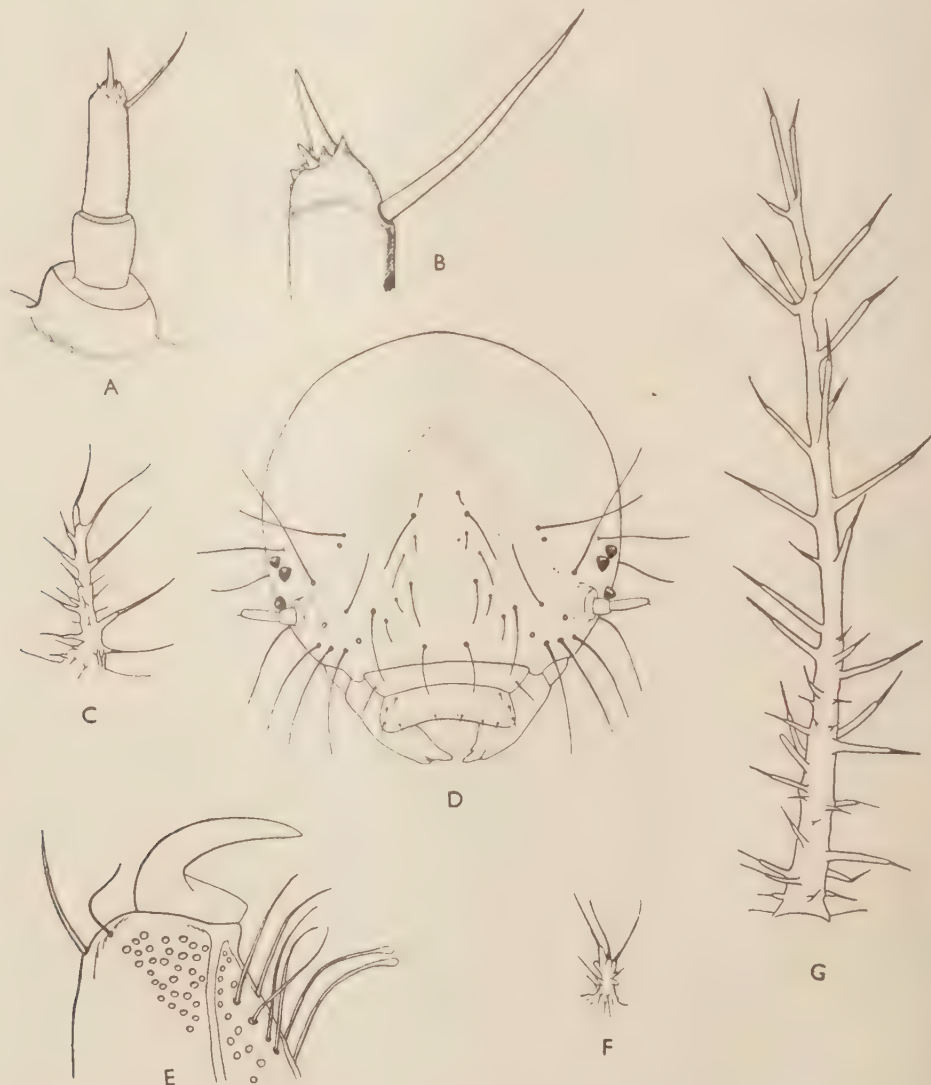


Fig. 18.—Larva of *Afissa dumerili*: (*a*) antenna ; (*b*) apex of antenna much enlarged ; (*c*) dorsolateral scolus of sixth abdominal segment ; (*d*) head ; (*e*) claw ; (*f*) dorsolateral scolus of seventh abdominal segment ; (*g*) subdorsal scolus of metathorax.

two-segmented, the basal segment a little longer than wide, the second a little narrower, about three times as long as wide, with a long seta situated a little below the apex and a small number of sensillae present at the apex, one of which is thinner and longer than the rest; clypeus relatively short, almost four times as wide as long; labrum a little longer than clypeus; mandibles stout, as wide as long not much narrowed distally, each with three rather short and blunt, heavily sclerotised teeth; the other mouth-parts as in most other EPILACHNINAE.

Thorax slightly increasing in diameter posteriorly. Prothorax less than twice as wide as long; pronotum almost uniformly sclerotised, rounded posteriorly; dorsal scoli directed anteriorly and a little upwards, nearly twice as long as pronotum, very narrow, each with 30 to 36 very short to rather long irregularly dispersed branches, with moderately long to short setae; subdorsal scoli absent; dorsolateral scoli about two-thirds the length of the dorsal, but with the branches more crowded, a few short chalazae present in the middle and along the posterior margin of the pronotum. Mesothorax a little wider than prothorax but shorter; the area of the tergum round the bases of dorsal and subdorsal scoli on each side and a little towards the middle of the segment sclerotised and provided with a few short chalazae; dorsal and subdorsal scoli of the same side very close together at their bases, diverging distally, directed a little anteriorly and each longer than the dorsal ones on the pronotum; dorsolateral scoli directed anterolaterally, equal to two-thirds the length of the dorsal scoli of the same segment, with about 28 comparatively shorter branches. Metathorax similar to mesothorax (fig. 18 g). On the underside, the ventral group on each thoracic segment consisting of two to four very short setae; coxae of any one segment well separated, femora slightly shorter than tibiae, claw (fig. 18 e) with a rather triangular tooth at base and moderately bent distally.

Abdomen with the first four segments nearly equal in width to the metathorax, the rest narrowing gradually posteriorly; dorsal scoli on the first six segments similar in structure to the corresponding scoli on the metathorax but a little shorter, on the seventh equal to nearly half the length of the dorsal scoli on the sixth, with relatively shorter and fewer, usually 16 branches, on the eighth like that on the seventh but half as long; subdorsal scoli on the first five segments equal in length and similar in branching to the dorsal scoli of the same segments, on the sixth (fig. 18 c) and seventh a little shorter, each with about 18 relatively shorter branches, on the eighth similar to the dorsal scoli on the same segment; dorsolateral scoli much shorter than the others of the same segment, usually directed laterally but in the last few segments directed successively more posteriorly, equal to two-thirds the length of the other scoli of the first three segments, and each with about 20 short, seta-bearing branches, but on the fourth to seventh (fig. 18 c, f) segments gradually decreasing in length, and on the eighth present in the form of a conical process bearing a few setae. The ninth segment with tergum short, semicircular, with a small struma on either side and with about eight setae on the posterior margin. On the ventral surface the setae poorly developed, short, almost transversely arranged and not arising from sclerotised areas; ventral group comprising usually three setae (one shorter) on each segment; subventral absent on first segment, consisting of two setae each on the second to fifth and of three on the sixth to eighth; ventrolateral group usually absent on the first, but sometimes comprising a single simple seta like that of the second, on the third and fourth segments consisting of a short chalaza and two short setae, on the fifth to seventh present in the form of strumae, each with about six setae, and on the eighth consisting of three simple setae. Ninth segment with six setae on either side; tenth without any setae.

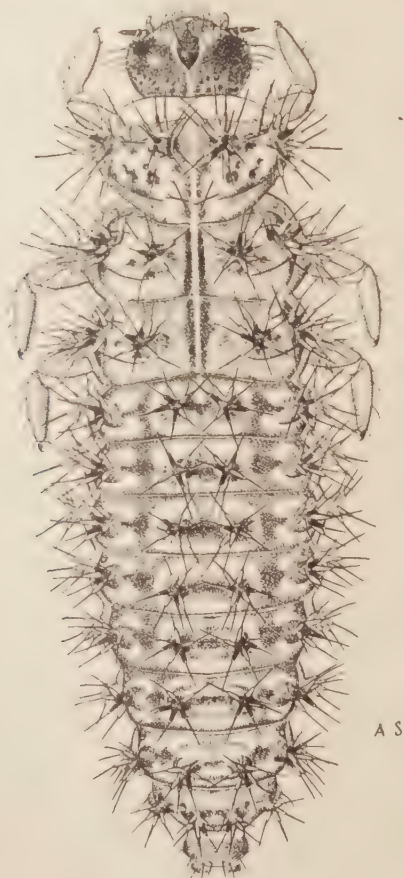
Material examined: 2 larvae from Jalpaiguri, North Bengal, feeding on *Clerodendron infortunatum* (Verbenaceae); February 1931, adults reared.

Genus *CHNOOTRIBA* Chevrolat *in* Dejean.

Chnootriba was first defined by Mulsant (1850) who regarded it as very distinct from the rest of the EPILACHNINAE. Weise, in 1898, however, sunk it as a synonym of *Epilachna* and since that time, until recently, the validity of this genus has remained uncertain. In Korschefsky's catalogue (1931), for instance, it was regarded as a subgenus of *Epilachna*. Mader, in 1941, considered it to be a valid genus and the present study of the larvae of the type species, *C. similis* (Thnb.), lends support to this view. The species feed mainly on Gramineae and the structure of the larvae is very distinct from most other members of the subfamily. Some of the differences have been mentioned in the key to the genera, while others are given in the description of the species in the following pages. Since larvae of the other species of the genus are not known, it is not possible to say which of these characters would be of greater value in separating it from the other genera of the subfamily.

***Chnootriba similis* subsp. *tellini* Weise (Figs. 19 and 20).**

Chnootriba similis occurs in Africa (except probably the south) and in south-west Arabia. It is divisible into several subspecies; that occurring in Abyssinia, Eritrea and south-west Arabia, being called *tellini* Weise. It is a serious pest of maize,



A S

Fig. 19.—Larva of *Chnootriba similis* subsp. *tellini* (relaxed).

sorghum, wheat and other related crops in these countries. Recently Jannone (1941) has given an account of its biology and control in Abyssinia. The larval material described here was reared by Mr. G. de Lotto at Asmara, Eritrea.

Body elongate oval, widest in the middle, 5.5–6.5 mm. long and 2.5–2.7 mm. wide; coloration very variable especially on the dorsal surface, head piceous to brown, thorax with elongate piceous markings in the middle and on the lateral parts of the tergites, abdomen piceous but with lighter areas round the bases of the scoli, which are also mostly piceous or brown but sometimes whitish, the underside light brown or



Fig. 20.—Larva of *Chnootriba similis* subsp. *tellini*: (a) branched subdorsal scolus of pronotum; (b) head; (c) unbranched subdorsal scolus of pronotum, much enlarged and with distal part of seta not shown; (d) antenna; (e) apex of antenna, much enlarged; (f) hypopharynx; (g) surface of the body-wall much enlarged; (h) maxilla and labium; (i) galea (ventral view) much enlarged; (j) mandible; (k) claw; (l) labrum; (m) subdorsal scolus of second abdominal segment; (n) dorsal scolus of pronotum of first-instar larva.

greyish in the middle, piceous or slightly paler on the lateral parts and round the bases of the setae in the median part.

Head (fig. 20 *b*) subrounded, vertex (except coronal suture), median part of front, the area anterior to the antennal sockets, ocelli and antennal segments piceous, other parts of head brownish; setae mostly long, and usually arranged as shown in the figure; ocelli three on either side and arranged triangularly, the two towards the vertex very close together and further from the third than from one another, the third situated outside and close to the antennal socket; antenna (fig. 20 *d*) two-segmented, long, with the first segment very short and about twice as wide as long, second long, about twice as long as wide, slightly narrowing in distal half, with a long seta at two-thirds and a few sensillae at apex (fig. 20 *e*); median part of clypeofrontal margin formed into a strongly sclerotised, triangular area with the apex towards the centre of the front and bearing a number of short setae; clypeus short and broad, trapezoidal, narrowing anteriorly, the lateral and anterior parts membranous, the former with about three setae on each side; labrum (fig. 20 *f*) rectangular, a little less than twice as wide as long, with very short but stout setae; mandibles (fig. 20 *g*) resembling those in *Cyngetis impunctata*, much longer than broad and narrowed distally, the base oblique on inner side, the condyle situated rather laterally, with four teeth, the apical one being large and bluntly denticulate on the inner margin; hypopharynx (fig. 20 *f*) emarginate in the middle and with moderately long setae on either side; maxilla (fig. 20 *h*) with the galea elongate, strongly sclerotised (rather horny) on the inner margin (fig. 20 *i*) and bearing dense, short setae distally, palpi relatively short, with the apical segment a little longer than the preceding and narrowed distally; labium (fig. 20 *h*) with partially sclerotised mentum and with narrow, moderately long, palpi.

Thorax (fig. 19) slightly widening posteriorly. Prothorax nearly twice as broad as long with the pronotum oval and slightly more rounded posteriorly; dorsal scoli very short, equal to half the length of the pronotum, broadly conical, each bearing about a dozen filiform branches arranged in the form of a rosette, each with a rather slender apical seta slightly longer than itself; the surface of these branches and of the body-wall (fig. 20 *g*) wrinkled except where strongly sclerotised; subdorsal scoli (fig. 20 *c*) small, each like a large branch of the other scoli and bearing a long apical seta (sometimes (fig. 20 *a*) there is a small branch in the middle which also bears a small seta at its apex); dorsolateral scoli similar to dorsal, slightly larger, each having about 16 branches. Mesothorax with the spiracles smaller than in most other species of the subfamily; dorsal scoli situated midway between the median longitudinal line of the segment and the subdorsal scoli, a little smaller than the corresponding one on pronotum, usually with eight branches; subdorsal larger than dorsal, with about ten branches similar to those of the dorsal; the area round bases of dorsal and subdorsal scoli of the same side more strongly sclerotised, oval and dark brown to piceous on the margins; dorsolateral scoli conical, directed rather laterally, each with about 12 branches similar to those of the others but usually brownish, seldom piceous. Metathorax with the scoli similar to those of mesothorax. On the underside, each segment with a single lightly piceous, subrounded struma situated in the middle of the sternum, that on the prosternum being small, and with about four, sometimes six, short setae, those on the meso- and metasternum being a little larger and each with six to ten short setae. Legs with tibia a little longer than femur, claw (fig. 20 *k*) without a quadrate basal tooth, rather triangular at base and gradually narrowed and pointed distally.

Abdomen with the spiracles relatively smaller than in most other species of the subfamily; dorsal scoli on each segment closer to one another than to the subdorsal, surrounded by a common transverse-oval strongly sclerotised area which is usually dark to piceous, but when lighter has a pair of pitted dark spots in the middle; dorsal scoli on first five segments similar, slightly smaller than the corresponding ones on metathorax, each with seven or eight branches bearing apical setae slightly

longer than themselves, the scoli on the sixth to eighth segments similar but with the base more conical ; subdorsal scoli (fig. 20 *m*) situated in line with the corresponding ones on metathorax but a little smaller, each bearing eight or nine branches except on the seventh and eighth segments where it is small and has only five or six branches ; dorsolateral scoli smaller than the subdorsal of the same segment and decreasing in size in each succeeding segment after the third ; on the first three segments, each with about seven branches, on the fourth to fifth segment with four or five branches and on the seventh and eighth more or less like a struma bearing six setae. Ninth tergite semicircular, sclerotised and pigmented distally, with about eight moderately long setae on either side and on the posterior margin. The ventral surface with the strumae sclerotised and dark, on the first segment the pair of ventral strumae usually absent (sometimes present only as a single, median and small struma, with a pair of setae), on the next five segments the strumae present in pairs, one on either side of the median longitudinal line and each with six to ten rather short setae, the seventh and eighth segments usually with about four setae ; subventral struma absent on first segment, on the succeeding segments similar to the ventral strumae of the same segment, on the eighth contiguous with the ventral ; ventrolateral strumae absent or represented by a single short seta on each of the first two segments, on the next six segments each struma with three to five setae. Ninth segment with a total of six to eight setae, present in a transverse median line; tenth with four very short setae on either side.

The characters of the earlier instars are as follows :—

First instar.—0.8–1.2 mm. long, 0.6–0.9 mm. wide, with relatively large head and long legs. Head subrounded, slightly broader than long, usually lighter than the rest of the body which is mostly piceous ; coronal and frontal sutures distinct, lighter in colour ; setae long, usually as in the subsequent instars ; of the ocelli the two towards the vertex relatively more distant from each other than in the fourth (final) instar larva ; antenna two-segmented as in the latter, with the first segment shorter than broad, the second not much narrower than the first, nearly as broad as long, with the seta and sensillae relatively more prominent ; the median triangular part of the antero-clypeal margin relatively smaller than in the final instar. Prothorax as wide as head and half as long ; dorsal scoli on pronotum situated more laterally than in the subsequent instars, each with about five short branches arising from a moderately long stem (fig. 20 *n*), each branch with the apical seta equal to about four times its length ; subdorsal scoli comprising a single filiform process bearing a long seta ; dorsolateral similar to dorsal. The scoli on the rest of the thoracic and on the abdominal segments similar in general arrangement to those described for the final instar but each with usually three branches like those of the dorsal scoli on the pronotum. On the underside the number of setae very small being usually two or three in a struma.

Second instar.—3.0 mm. long, 1.3 mm. wide, with the thoracic and first few abdominal segments wider than the head which is similar in structure to that of the final instar larva. Dorsal scoli on pronotum also closer together than in first instar ; the number of branches on the dorsal scoli on the pronotum and on the first few abdominal segments usually eight and five, respectively, each branch with the setae relatively shorter than in the first instar.

Third instar.—4.5 mm. long, 1.8 mm. wide ; like the fourth instar in general coloration and structure of the body ; the dark stripe on either side of the mid dorsal line on the thorax becoming distinct for the first time in this instar ; the scoli relatively shorter but with the number of branches equal to that in the fourth-instar larva.

Material examined: Many larvae of each instar (also eggs, pupae and adults) from Asmara, Eritrea, 1947 (G. de Lotto), damaging leaves of maize, sorghum, and wheat, and also attacking *Medicago sativa*.

***Merma mediata* Kapur (Figs. 21 and 22).**

The larvae of the genus *Merma* Weise have not been described before. The present material from Kawanda, Uganda (East Africa) was collected by Mr. W. V. Harris.

Body (fig. 21) elongate oval, slightly more tapering posteriorly, 5.0 mm. long and 1.7 mm. wide in the middle, uniformly and moderately convex on the upper surface; greater part of head and pronotum, most of the dorsal and subdorsal scoli and the areas of the tergite around their bases, brown to dark brown in colour, underside usually much lighter but with the legs and strumae brownish.

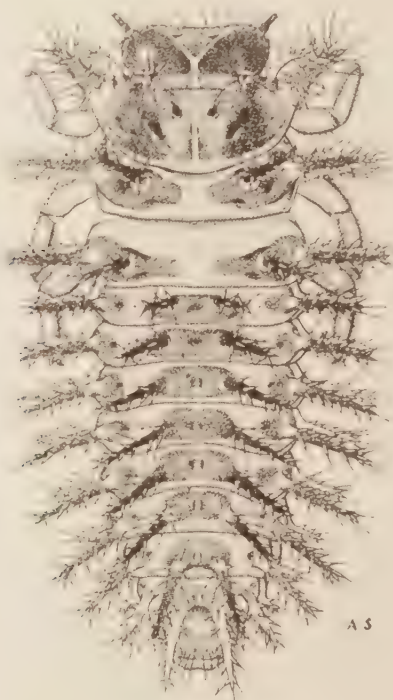


Fig. 21.—Larva of *Merma mediata*.

Head (fig. 22 a) larger in proportion to size of body than in other known genera, slightly broader than long, vertex and front dark brown except for the lighter epicranial suture comprising as usual a narrow coronal suture and a pair of frontal sutures, genae also lighter except for a dark area round the ocelli; setae moderately long to rather short, usually greyish and arranged as shown in the figure, ocelli three on each side, piceous, of equal size and placed triangularly as in most other species of the subfamily; antenna (fig. 22 c) long, two-segmented, dark brown, first segment slightly wider than long, second a little narrower and slightly tapering, twice as long as wide, with a moderately long seta situated a little below the apex and two longer and several short sensillae at the apex. Anterior margin of front with a small triangular, strongly sclerotised area in the middle; clypeus relatively large,

rather weakly sclerotised, trapezoidal, nearly twice as wide as long and with three setae on each lateral margin, labrum as wide as anterior margin of clypeus but slightly shorter, more strongly sclerotised at base and with about a dozen short setae; mandibles (fig. 22 *d*) only slightly longer than broad at base, the distal half with about seven teeth, the apical one largest and usually not denticulate on inner margin, three of the remaining six slightly larger than the other three and denticulate on the inner margin, hypopharynx with long and dense setae on the anterolateral angles; maxillae with the galeae subrounded, expanded, relatively large and with short setae instead of the usually long and dense ones present in most other EPILACHNINAE studied, maxillary palpus moderately long, with the apical segment much narrowed distally.

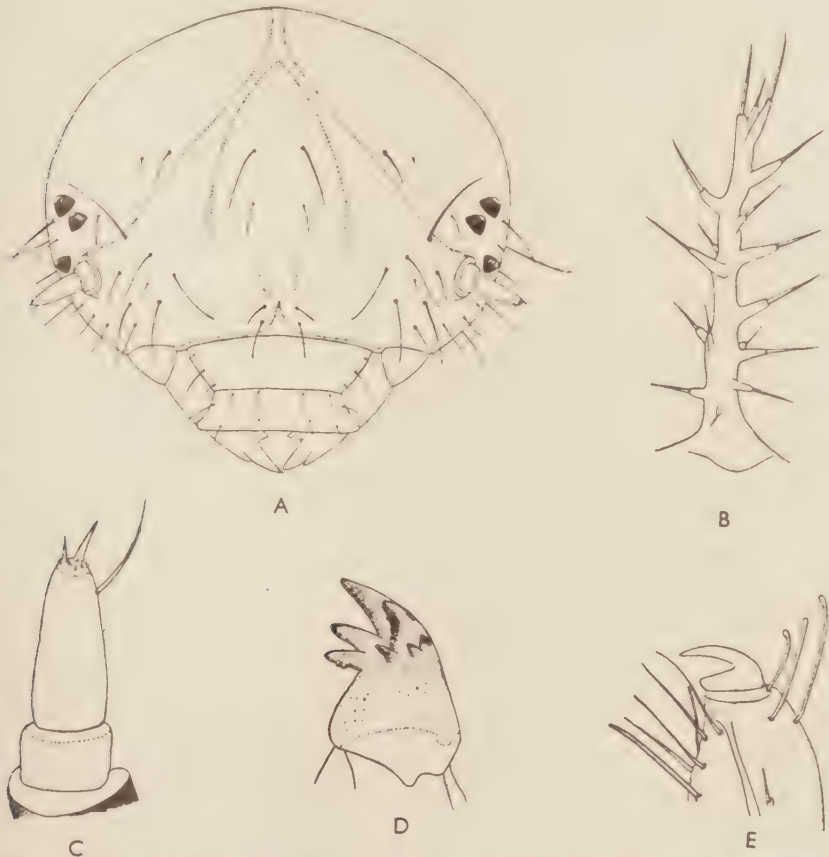


Fig. 22.—Larva of *Merma mediata*: (a) head; (b) subdorsal scolus of second abdominal segment; (c) antenna; (d) mandible; (e) claw.

Thorax slightly widening towards abdomen, slightly convex dorsally. Prothorax about twice as wide as long, pronotum oval, slightly more narrowed posteriorly, strongly sclerotised and pigmented irregularly brown to dark brown except for a very light and narrow longitudinal median line and four or five piceous patches on either side of it, dorsal scoli nearly as long as pronotum, the main stem usually piceous with about 20 branches arising from all over its surface, the branches usually lighter, short (equal to diameter of main stem), each with a thin apical seta usually

little longer than the branch bearing it, subdorsal scoli lighter in colour, sometimes asymmetrical, usually with a narrow main stem, equal in length to two-thirds the length of the dorsal scoli, and bearing four to six short branches similar to those on the latter but arising only on one side, dorsolateral scoli similar to dorsal but relatively broader at base and directed anterolaterally; there are also about a dozen short setae and chalazae on the rest of the surface of the pronotum. Mesothorax with the dorsal and subdorsal scoli of the same side with their bases near together and distinctly away from centre of segment, surrounded by a common strongly sclerotised brown to dark brown, subrectangular area; dorsal scoli slightly shorter and narrower at base than the corresponding scoli on pronotum, darker except at base, with about fourteen short branches bearing setae equal in length to themselves; subdorsal scoli (fig. 22 *b*) similar to dorsal but slightly longer and with about seventeen branches; dorsolateral scoli similar to dorsal except for its very light colour. Metathorax similar to mesothorax in general shape and armature. On the underside, each thoracic sternum with a pair of strumae, each bearing four to six moderately long setae. Legs dark brown for the most part, femora nearly as long as the tibiae, claw (fig. 22 *c*) with a subquadrate basal tooth, distal part moderately bent and sharply pointed.

Abdomen with the first three segments slightly wider than metathorax, the succeeding segments gradually narrowing posteriorly; the pair of dorsal scoli on each segment surrounded by an oval, sclerotised and brownish area; dorsal scoli on first five segments similar in coloration and size to those on metathorax but usually with the number of branches reduced to ten, on the sixth and seventh segment lighter, longer and each with 12 branches bearing setae as long as or slightly longer than themselves, setae on the eighth similar but much lighter in colour; subdorsal scoli each with a brown sclerotised area round the base, similar in structure to the dorsal ones of the same segment but shorter on the eighth; dorsolateral scoli without coloration except for a light brown area round the base of each, on the first four segments equal in length to one another and slightly shorter than the subdorsal ones of the same segments and each with about 15 shorter but more crowded branches, on the fifth to eighth segments decreasing rapidly in size, being only a conical projection bearing a chalaza and four short setae on the eighth. Ninth tergite relatively larger in size than in the other known larvae of the subfamily, subquadrate, the posterior margin slightly rounded, with a struma bearing about six short setae near each lateral margin and with about eight rather long setae on the posterior margin; tenth tergite slightly sclerotised distally, brownish and with a few very short setae. The ventral surface usually with small, light brown, rather ill-defined strumae with the setae becoming relatively longer in the last few segments; ventral strumae on the first eight segments each with usually three setae; subventral strumae absent on first segment and like the ventral strumae on the others; ventrolateral strumae with two short setae on first segment and four or five each on the succeeding seven segments, one of these setae borne on a pimple-like process. On the ninth segment, ten moderately long setae situated in a row in the posterior half of the segment; tenth with a few very short setae on either side.

Material examined: 5 larvae (several pupae and adults reared) 21.iii.1947, at Kawanda, Uganda (*W. V. Harris*).

Summary.

The EPILACHNINAE constitute one-sixth of the known species of the family COCCINELLIDAE. They are herbivorous and include a number of well known pests of cultivated plants in different parts of the world. Their adults present a great uniformity of external structure, with the result that nearly all the known species have been placed in one genus, *Epilachna* Hope. Their identification is made more

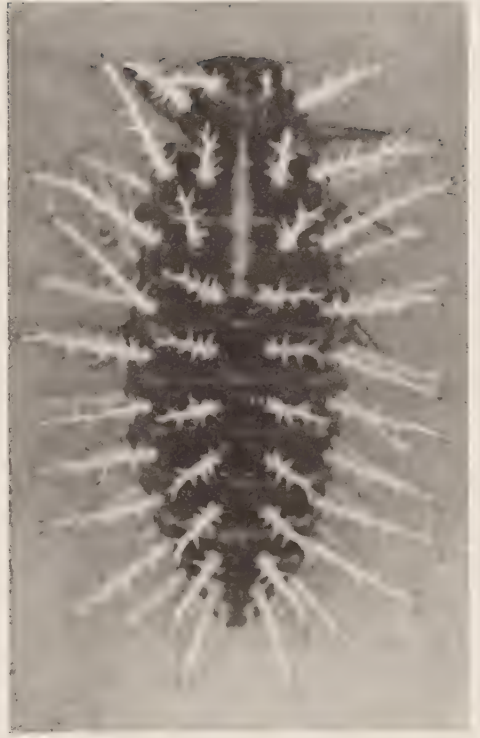
difficult by the employment of characters such as the elytral markings and spots which may vary a great deal in one species or be almost identical in two quite unrelated species. Lately, there has been a tendency to split the genus by employing more reliable morphological characters, including genitalia. A greater knowledge of the biology and morphology, including that of the immature stages, particularly the larvae, is necessary to evolve a natural classification. Relatively little was known of the larvae. This paper deals with 14 species belonging to six different genera.

In addition to discussion of the relationship of this subfamily with other COCCINELLIDAE in the light of the extensive larval material examined, it is observed that larvae belonging to different genera, including those recently erected or revived, show considerable morphological differences that support a division of *Epilachna* (s.l.) based on adult characters. A study of the larvae of nine species that are still retained in the genus *Epilachna* shows that they are separable into several groups, which may well indicate where further division of the genus may be made.

References.

- BÖVING, A. G. (1917). A generic synopsis of the Coccinellid larvae in the United States National Museum, with a description of the larva of *Hyperaspis binotata* Say.—Proc. U.S. nat. Mus., **51**, pp. 621–650, 4 pls.
- BÖVING, A. G. & CRAIGHEAD, F. C. (1931). An illustrated synopsis of the principal larval forms of the order Coleoptera.—Ent. amer., **11**, pp. 1–351, 125 pls.
- CANDÈZE, E. C. A. (1861). Histoire des métamorphoses de quelques coléoptères exotiques.—Mém. Soc. Sci. Liège, **16**, p. 40, pl. 6, fig. 8.
- CHAPUIS, F. & CANDÈZE, E. C. A. (1853). Catalogue des larves coléoptères connues jusqu'à ce jour.—Mém. Soc. Sci. Liège, **8**, pp. 1–313, 9 pls.
- CHUE, C. C. (1930). Some biological notes on a leaf-feeding Coccinellid (*Epilachna 28-punctata* Fabr.).—Lingnan Sci. J., **6**, pp. 301–313, 11 figs.
- DIEKE, G. H. (1947). Ladybeetles of the genus *Epilachna* (sens. lat.) in Asia, Europe and Australia.—Smithson. misc. Coll., **106**, no. 15, pp. 1–183, 27 pls., 6 figs.
- DIMMOCK, G. W. (1906). Algunas Coccinellidae de Cuba.—Inf. Estac. cent. agron. Cuba, **1**, pp. 287–292, 3 pls.
- DOEBNER, E. P. (1862). Beiträge zur Entwicklungsgeschichte einiger Coleopteren.—Berl. ent. Z., **6**, pp. 67–68.
- GAGE, J. H. (1921). The larvae of the Coccinellidae.—Ill. biol. Monogr., **6**, pp. 233–294, 6 pls.
- GANGLBAUER, L. (1899). Die Käfer von Mitteleuropa, **3**, 1046 pp., 46 figs.
- GORHAM, H. S. (1898). Biologia Centrali-Americana, Coleoptera, **7**, pp. 242–243, pl. 13, fig. 20.
- GRANDI, G. (1913). Studi sui Coccinellidi.—Boll. Lab. zool. Portici, **7**, pp. 288–292.
- HOWARD, N. F. (1941). Feeding of the Mexican bean beetle larva.—Ann. ent. Soc. Amer., **34**, pp. 766–769, 1 pl.
- HUBER, J. P. (1842). Mémoire pour servir à l'histoire de la Coccinella de la Saponaire.—Mém. Soc. phys. Genève, **9**, pp. 363–374, 1 pl.
- JANNONE, G. (1941). Osservazioni e rilievi su un singolare attacco di *Epilachna* (*Chnootriba*) *similis* ssp. *tellinii* Wse. (Coleoptera, Coccinellidae) alle colture di orzo e di frumento dell'Uollo Jeggiù (Scioa, A.O.I.).—Agricoltura colon., **35**, pp. 1–13, 63–73, 15 figs.

- KAPUR, A. P. (1944). On the biology and the structure of the Coccinellid *Thea bisoctonotata* Muls. in North India.—Indian J. Ent., **5**, pp. 165–171, 2 figs.
- KLEMM, M. (1930). Beitrag zur Morphologie und Biologie der *Epilachna chrysomelina* Fabr. (Coleopt.).—Z. wiss. Insektbiol., **24**, pp. 238–245, pl. 3–4, fig. 8–11.
- KORSCHESKY, R. (1931). Coleopterorum catalogus. Coccinellidae I. Heft 118, 224 pp.
- KRISHNAMURTI, B. (1932). The potato *Epilachna* beetle *Epilachna vigintioctopunctata* (Fabr.).—Bull. Dep. Agric. Mysore, Ent. Ser., No. 9, 16 pp. 5 pls.
- LEFROY, H. M. & HOWLETT, F. M. (1909). Indian Insect Life, pp. 308, 309. Calcutta, Thacker, Spink & Co.
- MADER, L. (1941). Coccinellidae. I. Teil.—Explor. Parc nat. Albert: Miss. de Witte (1933–35). Brussels, fasc. 34, 208 pp., 501 figs.
- MARRINER, T. F. (1927). Observations on the life history of *Subcoccinella 24-punctata*.—Ent. mon. Mag., **63**, pp. 118–123, 1 fig.
- MULSANT, E. (1846). Histoire naturelle des Coléoptères de France, **4**, Sulcicolles; Sécuripalpes, pp. 1–28 pl. 1, fig. 18. Paris.
- MULSANT, E. (1850–51). Species des Coléoptères trimères sécuripalpes.—Ann. Soc. Agric. Lyon, (2) **3**, pp. 1–1104.
- MULSANT, E. (1853). Opuscles entomologiques, pt. 3, p. 248.
- REDTENBACHER, L. (1843). Tentamen dispositionis generum et specierum coleopterorum pseudotrimerorum Archiducatus Austriae. Dissert., Vindobonae.
- SCHMIDT, E. (1922). Festschr. 50 j. Jubil. Lehranst. Obst- u. Gartenb. Geisenheim, pp. 512–514.
- SHARP, D. & MUIR, F. (1912). The comparative anatomy of the male genital tube in Coleoptera.—Trans. ent. Soc. Lond., **1912**, pp. 477–642, 37 pls.
- STROUHAL, H. (1927). Die Larven der palaearktischen Coccinellini und Psylloborini (Coleopt.).—Arch. Naturgesch., **92** (1926), Abt. A, pt. 3, pp. 1–63, 15 figs.
- WEISE, J. (1898). Coccinelliden aus Kamerun.—Dtsch. ent. Z., **1898**, pp. 97–125.
- WESTWOOD, J. O. (1839). An introduction to the modern classification of insects, I, pp. 395–398, fig. 49, no. 22. London.
- WIEDEMANN, C. R. W. (1823). Zweihundert neue Käfer von Java, Bengalen und dem Vorgebirge der guten Hoffnung.—Zool. Mag., Altona, **2**, pp. 1–133.
- ZIMMERMANN, K. (1936). Die geographischen Rassen von *Epilachna chrysomelina* F. und ihre Beziehungen zu *Epilachna capensis* Thunbg.—Z. indukt. Abstamm. u. VererbLehre, **71**, pp. 527–537, 2 maps, 11 figs.
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(a) (b) *Epilachna flavofasciata*.



(c) (d) *Epilachna dentulata*.

OBSERVATIONS ON THE BITING-HABITS OF SOME TABANIDAE IN UGANDA, WITH SPECIAL REFERENCE TO ARBOREAL AND NOCTURNAL ACTIVITY.*

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Observations on mosquitoes and other biting Diptera have been in progress in Bwamba County, Uganda, since early in 1942. Bwamba lies in the extreme west of Uganda and is to a large extent cut off from the rest of the country by the North Spur of the Ruwenzori Mountains (Mountains of the Moon). The country is low-lying, humid and rather hot, and is largely covered by uninhabited rain-forest. A detailed description of the area has been given elsewhere (Haddow, 1945a), to which reference should be made for further information on topography and vegetation.

Yellow fever is endemic in Bwamba County, both among the African population and among the wild monkeys. We have concentrated mainly on uninhabited rain-forest in search of the vector or vectors responsible for its transmission among the monkeys of this area. In 1944 yellow fever virus was isolated from a mixed group of *Aedes* spp. taken in uninhabited swamp-forest at Mongiro in Bwamba (Smithburn & Haddow, 1946). Mongiro lies in the north-eastern part of the Semliki Forest, which is continuous with the main Ituri Forest of the Congo Basin. Following the isolation of virus, work in this area was at once intensified in the hope of incriminating a single species of *Aedes*. This occupied most of our attention till the middle of 1945. As the occurrence of immunity among species of monkeys, such as *Cercocebus* and *Colobus* spp., which rarely descend to the ground, had led us to the conclusion that the vector of the disease among monkeys must be to a large extent arboreal (Haddow & others, 1947b), much of this work was carried out on platforms in trees. The methods used and the results of observations on mosquitoes have been given elsewhere (Haddow & others, 1947a; Haddow & Mahaffy, 1949), but a brief summary of the techniques employed is necessary for purposes of the present paper.

Following the isolation of yellow fever virus in 1944, platforms were built in trees at Mongiro at 54 feet in the main canopy, at 31 feet and at 16 feet. Working simultaneously at all levels, three of us (A. J. H., J. D. G. and R. B. H.) conducted a series of ten continuous 24-hour catches of biting Diptera. Units of four boys to each platform and a control unit of four boys at ground level were used. During these catches, made in June and July 1944 (wet season), all biting Diptera were recorded by hour and level. The forest at Mongiro is very swampy, and represents an edaphic climax. It forms a consociation dominated by *Mitragyna stipulosa* (DC.) O. Ktze. The most prominent among other species of trees are the oil-palm (*Elaeis guineensis* Jacq.) and *Voacanga obtusa* K. Schum. By way of contrast, and in order to increase the chances of isolating yellow fever virus, a second set of platforms was erected in an adjacent forest area at Mamirimiri, where the ecological background is quite different. Mamirimiri is an example of the local climatic climax, which culminates in a consociation dominated by the African ironwood (*Cynometra alexandri* C. H. Wright). This forest area has a higher canopy than that at Mongiro and much drier soil. It is contiguous, however, with an extensive swamp where the screw-pine

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(*Pandanus chiliocarpus* Stapf.) occurs in almost pure stand, interspersed with a few clumps of wild date (*Phoenix reclinata* Jacq.). The platforms at Mamirimiri were at 82 feet in an emergent tree, somewhat above the main canopy, at 58 feet in the main canopy, at 44 feet and at 22 feet. Here an identical series of ten catches was carried out, concurrently with the work at Mongiro. The species of trees in which platforms were built are listed below :—

MONGIRO

16 feet	<i>Macaranga schweinfurthii</i> Pax.
31 and 51 feet	<i>Cynometra alexandri</i> C. H. Wright
54, 56 and 58 feet	<i>Mitragyna stipulosa</i> (DC.) O. Ktze.
55 and 59 feet	<i>Kigelia moosa</i> Sprague

MAMIRIMIRI

22 and 44 feet	<i>Erythrina excelsa</i> Baker
58 feet	<i>Ficus</i> sp. indet. (Originally epiphytic ; a "strangler".)
82 feet	<i>Alstonia congensis</i> Engl.

Early in 1945, at the height of the dry season, one of us (A. J. H.) made a further series of ten catches on the same platforms in both areas, to investigate the behaviour of mosquitoes and other biting Diptera during hot dry weather.

Later in the same year, from April till June inclusive, two of us (A. J. H. and A. F. M.) carried out a long series of catches on tree-platforms at Mongiro and Mamirimiri in a continued search for the forest vector of yellow fever (Haddow & Mahaffy, 1949). These catches were carried out in the sunset period, as the mosquitoes desired were species whose main biting-activity occurs at that time. The experimental platforms at Mamirimiri and the 54-foot platform at Mongiro were again brought into use, and in addition four new platforms were constructed for this work at Mongiro, all in the main canopy, at 55, 56, 58 and 59 feet respectively. At first a unit of two boys to each platform was used, and catches were made on five days a week. Later, in order to increase the yield, the unit was increased to three boys, catches were made on six days a week, and an additional platform at 51 feet was built at Mongiro. The catches of this series were always started well before sunset, beginning at 16 to 17 hours local mean time, and were continued till well after dark, ending at 20.30 to 21 hours.

The purpose of the present paper is to discuss the Tabanids taken during this work, and more particularly to give some account of the biting habits and vertical distribution of a single species, *Chrysops centurionis* Austen, which was found to be essentially arboreal and nocturnal. Observations on arboreal Tabanids are still in progress in Bwamba and also in other parts of Uganda. The results of the work carried out since 1945 will form the subject of a separate paper.

Observations on *Chrysops centurionis*.

At Mongiro in the wet season of 1944, only one specimen of *C. centurionis* was taken at 54 feet between 23 and 24 hours. At Mamirimiri, on the other hand, 74 females were taken in the wet-season catches (Table I). All but three of these were taken on the two highest platforms, at the 58-foot and 82-foot levels. This was an unexpected finding. Still less expected were the observations that *C. centurionis*, first becoming noticeably active during the twilight period, shows a very distinct peak of biting-activity around sunset and that specimens may be taken biting throughout the night.

TABLE I.
Catches of *Chrysops centurionis* at Mongiro and Mamirimiri, wet season 1944 and dry season 1945.

Catches of <i>Chrysops centurionis</i> at Mongiro and Mamirimiri, wet season 1944 and dry season 1945.																														
Locality and Season	Level	Hours (Local Mean Time)																									Totals	Geo- metric means (A)	Days occur- rence (B)	Biting Index 100 AB
		Day															Night													
		06- 07	07- 08	08- 09	09- 10	10- 11	11- 12	12- 13	13- 14	14- 15	15- 16	16- 17	17- 18	18- 19	19- 20	20- 21	21- 22	22- 23	23- 24	00- 01	01- 02	02- 03	03- 04	04- 05	05- 06					
Mongiro, wet season 1944.	54'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	0.07	1	7	
	31'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	16'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	0'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	Totals	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	
Mongiro, dry season 1945.	54'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	31'	—	—	—	—	—	—	—	—	—	—	6	6	3	2	—	—	—	—	—	—	—	—	—	—	17	1.24	7	868	
	16'	—	—	—	—	—	—	—	—	—	—	2	2	3	—	—	—	—	—	—	—	—	—	—	—	7	0.37	3	111	
	0'	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	1	0.07	1	7	
	Totals	—	—	—	—	—	—	—	—	—	—	8	9	6	2	—	—	—	—	—	—	—	—	—	—	25	—	—	—	
Mamirimiri, wet season 1944.	82'	1	—	—	—	—	—	—	—	—	—	1	10	9	5	1	2	2	—	1	1	1	—	—	—	36	2.52	7	1,764	
	58'	—	—	—	—	1	—	—	1	1	1	2	19	5	1	2	1	—	—	1	—	—	—	—	—	35	3.31	10	3,310	
	44'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0		
	22'	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	1	—	—	—	3	0.20	2	40	
	0'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	Totals	1	—	—	—	1	—	—	1	1	1	4	30	14	6	3	3	—	1	2	2	—	1	3	74	—	—	—	—	
Mamirimiri, dry season 1945.	82'	—	—	—	—	—	—	—	—	—	—	—	3	1	—	—	—	—	1	—	—	—	—	—	—	5	0.37	4	148	
	58'	—	—	—	—	—	—	1	—	—	—	1	4	1	1	4	—	1	—	—	—	—	—	—	—	13	0.60	3	180	
	44'	—	—	—	—	—	—	—	—	—	—	—	3	3	1	1	—	—	—	—	—	—	—	—	—	8	0.43	3	129	
	22'	—	—	—	—	—	—	—	—	—	—	2	2	4	4	—	1	—	—	1	—	—	—	—	—	15	1.29	8	1,032	
	0'	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00	0	0	
	Totals	—	—	—	—	—	1	1	—	—	—	3	2	14	9	2	6	—	1	1	1	—	—	—	—	41	—	—	—	
Grand Total	1	—	—	—	1	1	1	1	1	1	3	14	53	29	10	9	3	2	2	3	2	—	1	3	141	—	—	—	
Geometric Mean (A)	0.02	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.05	0.25	0.85	0.43	0.16	0.14	0.05	0.04	0.04	0.05	0.04	0.00	0.02	0.05	—	—	—	—	
Days Occurrence (B)	1	—	—	—	1	1	1	1	1	1	2	6	10	8	5	5	2	2	2	3	2	—	1	1	—	—	—	—	
Biting-Index 100 AB*	2	0	0	0	2	2	2	2	2	2	10	150	850	344	80	70	10	8	8	15	8	0	2	5	—	—	—	—	

* The Biting-Index is a figure which in any given hour (or at any given level) takes into account both the number of specimens taken and the number of days on which they were caught. It consists basically of the product of the number of specimens and the number of days but, to minimise the effects of single unusually large or unusually small catches, the geometric means are used instead of the crude totals or arithmetic means. To give integer numbers the products are multiplied by 100.

In the dry-season catches of 1945, 25 specimens were taken at Mongiro, mainly at the 31-foot level, while 41 were taken at Mamirimiri, mainly at the 22-foot and 58-foot levels. The time of maximum biting-activity was again the sunset period and early night.

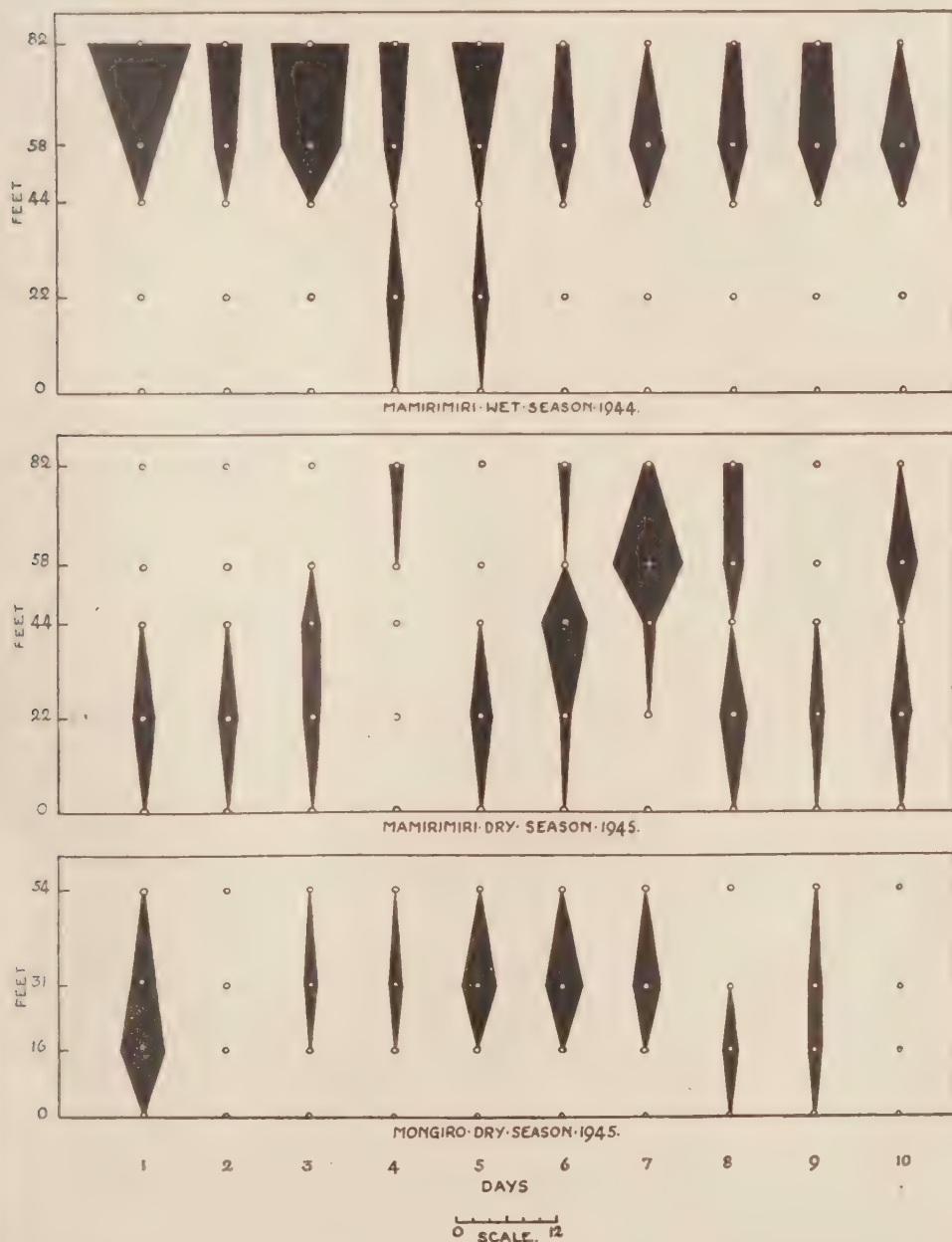


Fig. 1.—The vertical distribution of *C. centurionis* from day to day. Mongiro and Mamirimiri, 1944-45. Crude figures. (See Table I.)

The first point of importance is the unexpected vertical distribution of this species. In figure 1, which shows the vertical distribution from day to day, the following facts become apparent :—

(a) In the wet season of 1944 at Mamirimiri, *C. centurionis* exhibited a very definite preference for the 58-foot and 82-foot levels. At 58 feet in the main canopy, it was present on every night of the series of catches. At 82 feet in an emergent tree, it was present on seven of the ten nights concerned. Only on two occasions, in catches Nos. 4 and 5, was *C. centurionis* taken at levels under 58 feet, and then only in small numbers. In the wet season at Mongiro, the single specimen obtained was also taken in the canopy.

In the wet season of 1944, therefore, *C. centurionis* showed a well-marked preference for the upper levels of the forest.

(b) In the dry season of 1945 at Mamirimiri, the vertical distribution was markedly irregular, differing considerably from day to day (fig. 1). The crude figures show that *C. centurionis* preferred the 58-foot and 22-foot levels. At Mongiro it had a distinct preference for the 31-foot level.

In the dry weather, therefore, the vertical distribution of the species differed markedly from that observed in the wet season.

In considering the biting-activity of an insect, much information may be gained by a study of the total numbers taken at different levels. In cases where the transmission of an endemic disease may be involved, however, there is a second group of data which is of equal or even greater importance. This concerns the regularity of occurrence day after day. An insect which appears daily in moderate numbers is likely to be of more importance in the transmission of an endemic disease than one which, though it may occur in equal or larger numbers, is not active every day.

In this connection it was thought that a "Biting-Index", that is, a figure combining the numbers of specimens taken with the number of days on which they occurred, might be used profitably. In its first form, this figure was obtained simply by multiplying the total numbers taken biting at a given level by the number of days of their occurrence at that level. An obvious fallacy, however, was the fact that this crude figure was unduly influenced by unusually large catches made on single nights. To reduce the influence of these unusual catches on the final result, the geometric mean catch per night was substituted for the crude total, using the $\log(n+1)$ technique of Williams, 1937. The figures obtained were finally multiplied by 100 to give integer numbers, convenient for tabulation and study. The effect of the index is to concentrate attention on the level which combines numerical prevalence and regular occurrence in the greatest degree, and to minimise the importance of levels where numbers are large but occurrence irregular. To compare the results of one series directly with those of another, the indices for each series may be summed and expressed as percentages of their respective totals, providing samples are of a reasonable size. In the present instance, the use of the biting-index (Table 1 and fig. 2) shows that :—

(a) In the wet season at Mamirimiri the canopy at a height of 58 feet was the most important level. Next in importance was the zone of emergent trees above the canopy at a height of 82 feet. It is regrettable that *C. centurionis* was so scarce at Mongiro during this season, but it is again noted that the single specimen was taken in the canopy, at 54 feet.

(b) In the dry weather at Mamirimiri the important zone was at the 22-foot level, all others being of very minor significance. In the same season at Mongiro, the 31-foot level was the only important zone.

These results indicate that in the wet season *C. centurionis* was most prevalent in the canopy, and in the dry season at a height intermediate between ground-level

and the canopy. This suggests that it prefers a good deal of shelter. In the wet season, the canopy was very dense and the evenings were, on the whole, calm. In the dry season, the canopy foliage was very sparse and the weather was exceedingly hot. In addition, strong katabatic breezes were very frequent in the sunset period during the dry season of 1945. These had much more influence on the forest canopy than on the more sheltered lower levels.

The seasonal incidence of *C. centurionis* has not been clarified by subsequent work; and when beginning a series of catches, it was never certain whether or not this species would be present, even in localities where previous observations have shown it to be prevalent. This seems to indicate that, like many other Tabanids, it may have a very prolonged life-cycle, and so be less influenced by seasonal changes of short duration than are some other groups such as, for example, mosquitoes.

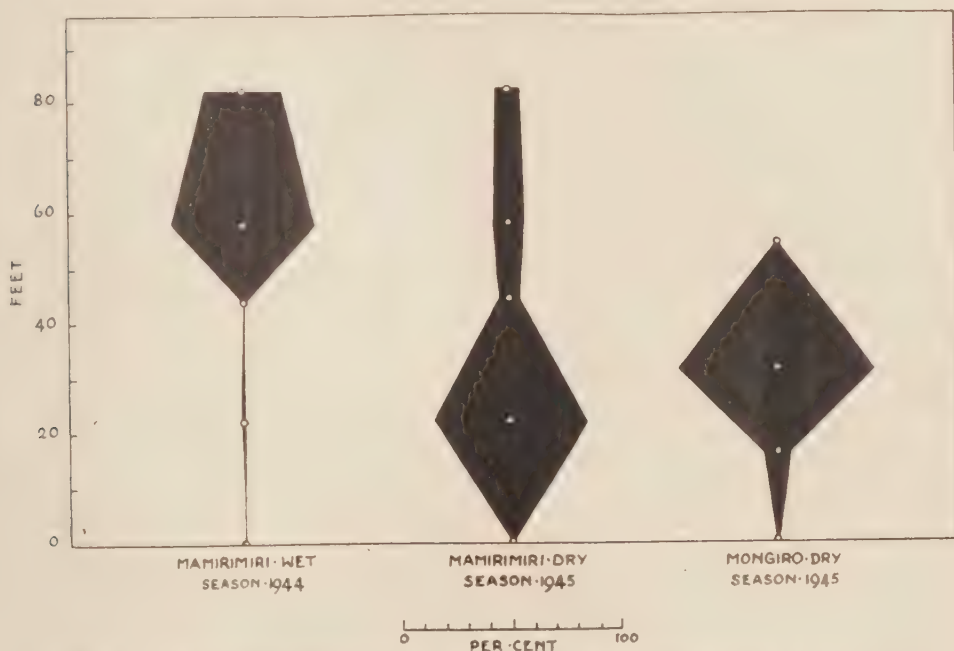


Fig. 2.—The vertical distribution of *C. centurionis* in different seasons, as shown by the biting-index, $100M_G \times \text{days' occurrence}$. (See Table II.)

The biting-cycle of *C. centurionis* may now be considered. The catch of one specimen at Mongiro in 1944 may be ignored in much of the discussion. Examination of the crude data in Table I shows that, in general, biting begins shortly before sunset at 17–18 hours, that it reaches a distinct peak in the hour after sunset at 18–19 hours, and thereafter falls away gradually. In most cases the numbers taken after 20 hours were small. The greatest scatter of observations occurred at the levels where the largest numbers of specimens were taken, while at levels where only small numbers occurred the catch was more clearly concentrated in the most favourable period around sunset. It will further be noted that at the 82-foot level at Mamirimiri only two specimens, or 5 per cent. of the total for this level, were taken by day in the 20 catches made in this area, while at the 58-foot level eight specimens, or 17 per cent. of the total, were taken by day. This clearly reflects the rather open and exposed nature of the 82-foot platform.

In making a comparison between the four series by levels, it seems better to use the hourly geometric mean catches than the crude figures (Table II). This table shows that in the dry season at Mongiro the hour before and the hour after sunset, 17-18 and 18-19 hours respectively, were the times of greatest activity, the differences between these hours being small and probably not significant. In the dry season at Mamirimiri, the peak at 82 and 58 feet occurred between 18 and 19 hours, and at 44 and 22 feet between 18 and 20 hours. In the wet season at Mamirimiri, the two hours after sunset, 18-20 hours, were those during which most activity occurred, with the first hour after sunset showing a well-marked peak of biting-activity at the 58-foot level.

The main biting-activity of *C. centurionis*, therefore, falls within the period covered by the hour before sunset and the two hours after sunset. The specimens taken before 18 hours usually bit within the last 15 minutes of the preceding hour. Subsequent work, which will be reported separately, confirms these observations, and emphasises that the hour after sunset is by far the most important.

As the variation from series to series and from level to level is not very marked, the biting cycles for all levels and for both seasons combined give a very fair picture of the activity of *C. centurionis*. In calculating the general summary of the biting-cycle, it seems best to use geometric means (Table I), although the fact that the general trend of the geometric means follows closely that of the crude totals indicates that the results were very consistent from day to day.

As in the case of vertical distribution, a biting-index may be worked out by finding the product of the hourly geometric means and the number of days on which specimens were taken at each hour, and multiplying the result by 100. The index (Table I) serves to emphasise still further the importance of the period 17-20 hours, and more particularly of the period 18-19 hours.

The foregoing observations indicate that *C. centurionis* shows highly specialised biting-behaviour. In the case of mosquitoes, it has been found that species, such as *Taeniorhynchus* (*Mansonioides*) *africanus* (Theo.), which are prevalent in many kinds of habitat, usually have shown very variable biting-cycles, and will freely attack several quite unrelated hosts—man, monkeys, dogs, birds and probably others, while mosquitoes which are markedly selective with regard to habitat, such as *Aedes* (*Mucidus*) *grahami* (Theo.), *A. (Finlaya) longipalpis* (Grünb.) and *A. (Stegomyia) africanus* (Theo.), show a clearly defined biting-cycle, centering on some particular period of the day or night (Haddow & others, 1947a). With regard to mosquitoes of the latter type, it has seemed reasonable to assume that the host preference is equally specialised, a fact that has been shown to apply in the case of *A. (S.) simpsoni* (Theo.). An experiment with man, baboons, goats and fowls showed that this species, the vector of yellow fever among the human population of Bwamba, prefers human blood (Haddow, 1945b). If this hypothesis be maintained, it seems justifiable to suggest that *C. centurionis* may also be rather specialised in its host-preference.

Direct observation has shown that, while *C. centurionis* will bite boys catching in trees with comparative freedom, man is obviously not the preferred host. So much, indeed, may be inferred from the fact that this species is comparatively scarce at ground-level. The general preference of Tabanids for mammalian blood would suggest that it probably feeds normally on arboreal mammals. The main groups which might be involved in Bwamba are primates, bats, squirrels, genets and tree-civets (*Nandinia*). Of these animals, those less likely to become natural hosts of *C. centurionis* are bats, genets, tree-civets and one section of the primates (the Lemuroidea), which are active by night, and squirrels, which sleep mainly in tree holes or under loose bark. This leaves, as the probable hosts, the monkeys, which all sleep in the open in trees in Bwamba, and which usually take up their positions

in their sleeping-trees before sunset and are asleep by the time that *C. centurionis* has begun to bite actively. Finally, experiments made in the canopy with two species of African monkeys have shown that, in its natural habitat, *C. centurionis* will bite monkeys freely in the presence of man (Haddow & Dick, 1948).

In this connection it is interesting to note that the nearest known allies of *C. centurionis* are *C. dimidiata* v.d. Wulp, and *C. silacea* Aust. The recorded differences are almost entirely in adult coloration (Austen, 1911). *C. dimidiata* and *C. silacea* are well-known vectors of the human filarial parasite *Loa loa*. Filarial infections

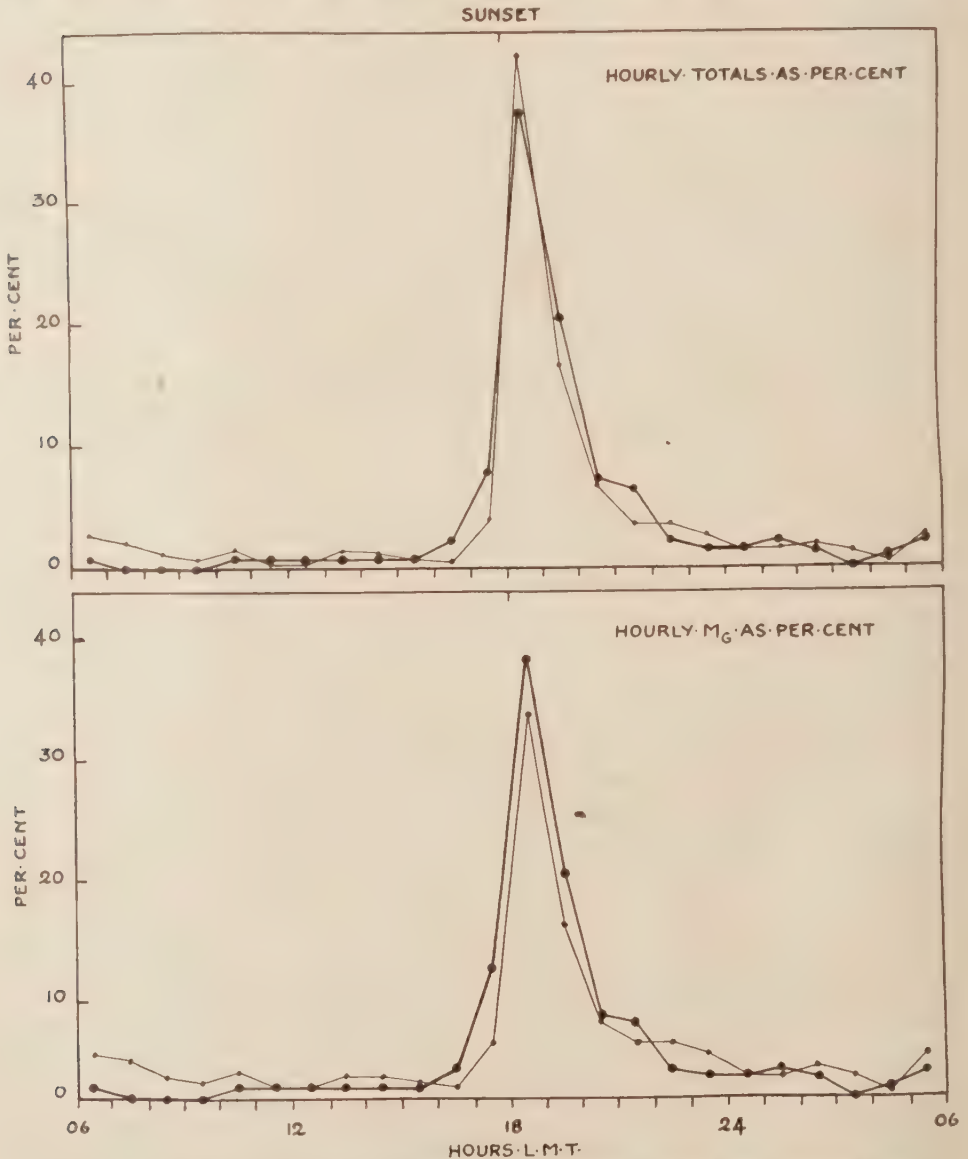


Fig. 3.—The biting-cycle of *C. centurionis* (thick lines with large spots) compared with that of *A. africanus* (thin lines with small spots), Mongiro and Mamirimiri, 1944-45. (See Table III.)

are known to occur commonly in at least five species of Uganda monkeys, and are probably most prevalent in the highly arboreal black mangabey, *Cercocebus albigena johnstoni* (Lydekker), which seldom descends to ground-level. It is suggested that *C. centurionis* may be involved in the transmission of such infections.

It is instructive to compare the biting-behaviour of *C. centurionis* with that of *Aedes africanus*, a mosquito which readily bites monkeys of various species in the forest canopy (Haddow & Dick, 1948). Both are prevalent in the canopy and both bite most actively at the same period. Figures for *A. africanus* taken in the Mongiro and Mamirimiri catches of 1944-45 have already been published (Haddow & others, 1947a). They are quoted here, with both the crude figures and the geometric means given, to allow comparison with figures for *C. centurionis* (Table III and fig. 3). The correspondence between the biting-behaviour of these insects is very striking, the coefficient of correlation between the two series of crude figures being remarkably high ($r = +0.971 \pm 0.012$). Simpson and Roe (1939) state that in zoological work the use of the standard error of r is liable to give misleading results where r is high. They suggest that the use of z and its standard error is preferable in such cases. In the present instance $z = 2.1 \pm 0.22$, a highly significant result. That the biting behaviour is very consistent in both cases is shown by the fact that the general trends of the crude figures and geometric means are very similar (fig. 3).

As the correspondence between the biting-behaviour of these entirely unrelated insects is so remarkably close, it seems reasonable to suppose that it may be controlled by the same factors. The suddenness with which both species became active leads one to believe that light must be the most important factor, as it is the only one which changes with sufficient rapidity in the sunset period. It is believed that temperature may play an important secondary part, for the sunset activity of *A. africanus* may be partly, or even completely, inhibited on cold evenings (Haddow & Mahaffy, 1949). This view is supported by the fact that neither species shows a marked peak of activity at dawn, when low light intensities again prevail but temperature is at its lowest for the entire 24-hour period. The third main micro-climatic factor, saturation deficiency, is probably unimportant, as it remains relatively constant and very low throughout the night in the Semliki Forest.

The discussion has so far been concerned with the 141 specimens taken in the 24-hour catches made in 1944 and early in 1945. The results of the long series of routine catches made at Mongiro and Mamirimiri later, from April to June, 1945, may now be mentioned briefly, as they provide further evidence of the arboreal and crepuscular habits of *C. centurionis*.

At Mamirimiri 58 catches, totalling 2,251 man-hours, were made on the four platforms between 22 to 82 feet above ground. As mentioned above, these catches were begun well before dark, at 16-17 hours, and continued till well after dark, 20.30-21 hours. They yielded a total of 278 *C. centurionis* and 3,062 mosquitoes. Thus *C. centurionis* represented about 8 per cent. of the total catch of biting Diptera.

Simultaneously, at Mongiro, 64 catches were made in the canopy on platforms between 50 and 60 feet above ground. At first five platforms were in use, but later the number was increased to six. These catches totalled 3,158 man-hours and yielded 261 *C. centurionis* and 3,776 mosquitoes. In this case, *C. centurionis* represented over 6 per cent. of the total Diptera taken in the canopy.

During the first two weeks at Mongiro, five catches per week were made on five high platforms, with a unit of two boys to a platform. Two boys acting as controls were stationed at ground-level below each platform. These catches totalled 300 man-hours in the canopy and 300 at ground-level. The platform catches yielded 150 *C. centurionis* and 301 mosquitoes. The ground-level catches yielded 17 *C. centurionis*, 9 *Glossina* spp. and 1,134 mosquitoes. During this period, therefore,

C. centurionis formed about 33 per cent. of the Diptera taken in the canopy, and less than 2 per cent. of those taken at ground-level. In this section it has been shown that *C. centurionis* is active after dark and that, while the majority of biting females are taken shortly after sunset, some activity goes on throughout the night. Males are also active after dark.

TABLE III.

The biting-cycles of *Chrysops centurionis* and *Aedes africanus* at Mongiro and Mamirimiri, wet season 1944 and dry season 1945, for comparison. Totals, geometric means, and percentages.

Hour (L.M.T.)	<i>Chrysops centurionis</i>				<i>Aedes africanus</i>			
	Hourly totals (No.)	Hourly totals (Per cent.)	Hourly geo- metric means (No.)	Hourly geo- metric means (Per cent.)	Hourly totals (No.)	Hourly totals (Per cent.)	Hourly geo- metric means (No.)	Hourly geo- metric means (Per cent.)
06-07	1	0.7	0.02	0.8	13	2.8	0.20	3.5
07-08	0	0.0	0.00	0.0	10	2.2	0.18	3.1
08-09	0	0.0	0.00	0.0	6	1.3	0.10	1.7
09-10	0	0.0	0.00	0.0	4	0.9	0.07	1.2
10-11	1	0.7	0.02	0.8	7	1.5	0.12	2.1
11-12	1	0.7	0.02	0.8	2	0.4	0.05	0.9
12-13	1	0.7	0.02	0.8	3	0.6	0.05	0.9
13-14	1	0.7	0.02	0.8	6	1.3	0.10	1.7
14-15	1	0.7	0.02	0.8	5	1.1	0.10	1.7
15-16	1	0.7	0.02	0.8	4	0.9	0.07	1.2
16-17	3	2.1	0.05	2.2	3	0.6	0.05	0.9
17-18	14	9.9	0.25	10.8	18	3.9	0.26	4.5
18-19	53	37.6	0.85	36.5	195	42.1	1.82	31.7
19-20	29	20.6	0.43	18.6	76	16.4	0.82	14.3
20-21	10	7.1	0.16	6.9	31	6.7	0.35	6.1
21-22	9	6.4	0.14	6.1	16	3.5	0.26	4.5
22-23	3	2.1	0.05	2.2	16	3.5	0.26	4.5
23-24	2	1.4	0.04	1.7	12	2.6	0.20	3.5
00-01	2	1.4	0.04	1.7	5	1.1	0.10	1.7
01-02	3	2.1	0.05	2.2	6	1.3	0.10	1.7
02-03	2	1.4	0.04	1.7	8	1.7	0.15	2.6
03-04	0	0.0	0.00	0.0	5	1.1	0.10	1.7
04-05	1	0.7	0.02	0.8	2	0.4	0.02	0.3
05-06	3	2.1	0.05	2.2	10	2.2	0.20	3.5

Below are notes on the nocturnal activity of *Haematopota vittata* (Lw.) and *H. nefanda* (Edw.). First, however, it may be mentioned here that one of us (A.J.H.) had taken other Tabanids after dark in the forest canopy at Bwamba and other parts of Uganda, namely: *Tabanus thoracinus* P. de B., *Haematopota* sp. indet., aff. *nefanda* Edw. and *Chrysops funebris* Aust. We have thus evidence of nocturnal activity in the case of six species, belonging to three separate genera. It is interesting to note that the significance of the remarkable eye-colours of the Tabanids has never been satisfactorily explained, nor has the fact that there is often an area in the upper part of the eye composed of facets larger than those in the lower part (Edwards & others, 1939). The species of *Chrysops* and *Haematopota* here mentioned have all more or less brilliantly banded eyes, while the males of *T. thoracinus* (the females have not yet been seen in life) have an upper area of dull greenish bronze, sharply demarcated from a lower area of very brilliant emerald green. It seems possible that, in some cases at least, the eye-colours of Tabanids may have some connection with crepuscular or nocturnal activity. Many species are known to bite almost entirely by day, but little as yet is known of the mating habits of this group, and of the fact that the eyes of the males are of quite exceptional size and brilliance.

Other Tabanids taken in 24-hour Catches.

Though *C. centurionis* was the only Tabanid taken in numbers in the catches at Mongiro and Mamirimiri, two other species were represented, as follows :

Tabanus xanthomelas Aust.

Mamirimiri, wet season 1944 ; one female, 82 feet, 17-18 hours.

Chrysops griseicollis Beq.

Mongiro, dry season 1945 ; one female, 31 feet, 14-15 hours. Mamirimiri, dry season 1945 ; one female, 22 feet, 16-17 hours ; one female, 58 feet, 12-13 hours.

It will be noted that all three specimens of the rare *C. griseicollis* were taken in the afternoon during the dry season.

Other Records of Bwamba Tabanids.

Apart from the foregoing more or less detailed observations, records of Tabanids taken during routine mosquito catches in Bwamba have been kept since 1942. They may be summarised as follows :

Tabanus williamsi Aust.

Biting, forest floor, by day. Scarce.

T. par Wlk.

Biting, forest floor and banana plantations, by day. Not uncommon.

T. ditaeniatulus Macq.

Biting, forest floor, by day. Scarce.

T. taeniola P. de B.

Biting, forest floor, by day. Scarce.

Haematopota vittata (Lw.)

Biting, forest floor and banana plantations, mainly by day but sometimes after dusk. Appears to be common on elephants. May be taken in numbers biting newly-shot elephants in the forest.

H. brucei (Aust.)

So far not taken biting man. Common on newly-shot elephants in the forest, with *H. vittata* and *Glossina* of the *fusca* group. Not yet seen on elephants shot outside the forest.

H. nefanda (Edw.)

Biting, forest floor, by day and by night. Scarce.

Hippocentrum strigipennis (Karsch).

Occasionally taken biting by day, forest floor and banana plantations. Exceedingly common in marshland just outside the main Semliki Forest (at Niansimbi Hot Spring). Bites viciously in this area. Hundreds may be taken in a few hours.

Chrysops longicornis v. *funnebris* Aust.

Biting, forest floor, by day. Scarce.

Summary.

In a search for the forest vector of yellow fever, catches of biting Diptera have been made by various methods in forest trees in Bwamba County, Uganda. The work here reported concerns Tabanids taken in catches made during the period 1944-45.

Three species of Tabanids have been taken in trees during these catches, and one of these, *Chrysops centurionis*, has proved to be mainly arboreal.

Observations on *C. centurionis* have shown that its main biting-activity begins just before sunset and reaches a peak during the hour after sunset. Thereafter the numbers taken diminish rapidly, but some activity continues throughout the night.

In the wet-season catches, this species was most prevalent in the forest canopy at heights of 50 to 60 feet above ground. In the dry season the most favourable level was lower, at 20 to 30 feet above ground.

It is suggested that monkeys are the natural hosts of *C. centurionis*, and that *C. centurionis* may be the vector of filarial infections among wild monkeys.

It is shown that there is a very close correspondence between the biting-behaviour of *C. centurionis* and that of the mosquito, *Aedes africanus*.

Evidence of nocturnal activity has now been obtained in the case of one species of *Tabanus*, three species of *Haematopota* and two species of *Chrysops*. It is suggested that there may be some connection between the remarkable eye colours of Tabanids and crepuscular or nocturnal activity.

Records of other Bwamba Tabanids are given.

Acknowledgements.

We wish to thank Dr. F. van Emden, of the Commonwealth Institute of Entomology, and Mr. H. Oldroyd, of the British Museum (Natural History), who have confirmed the identification of every species except *Tabanus xanthomelas*. Our thanks are also due to Dr. J. C. R. Buchanan, then Acting Director of Medical Services, Uganda Protectorate, and the late Dr. F. J. Johnstone, then Acting Director of Medical Services, Kenya Colony, by whose permission two of the authors were enabled to take part in the investigation.

References.

- AUSTEN, E. E. (1911). Three new African species of the genus *Chrysops* (family Tabanidae).—Bull. ent. Res., **2**, pp. 161, 168.
- EDWARDS, F. W., OLDROYD, H. & SMART, J. (1939). British blood-sucking flies. London, Brit. Mus. (Nat. Hist.).
- HADDOW, A. J. (1945a). On the mosquitoes of Bwamba County, Uganda. I. Description of Bwamba, with special reference to mosquito ecology.—Proc. zool. Soc. London, **115**, pp. 1-13.
- HADDOW, A. J. (1945b). The mosquitoes of Bwamba County, Uganda. II. Biting activity, with special reference to the influence of microclimate.—Bull. ent. Res., **36**, pp. 33-73.
- HADDOW, A. J. & DICK, G. W. A. (1948). Catches of biting Diptera in Uganda, with anaesthetized monkeys as bait.—Ann. trop. Med. Parasit., **42**, pp. 271-277.

- HADDOW, A. J., GILLET, J. D. & HIGHTON, R. B. (1947a). The mosquitoes of Bwamba County, Uganda. V. The vertical distribution and biting-cycle of mosquitoes in rain-forest, with further observations on microclimate.—Bull. ent. Res., **37**, pp. 301–330.
- HADDOW, A. J. & MAHAFFY, A. F. (1949). The mosquitoes of Bwamba County, Uganda. VII. Intensive catching on tree-platforms, with further observations on *Aedes (Stegomyia) africanus* Theobald.—Bull. ent. Res., **40**, pp. 169–178.
- HADDOW, A. J., SMITHBURN, K. C., MAHAFFY, A. F. & BUGHER, J. C. (1947b). Monkeys in relation to yellow fever in Bwamba County, Uganda.—Trans. R. Soc. trop. Med. Hyg., **40**, pp. 677–700.
- SIMPSON, G. G. & ROE, A. (1939). Quantitative zoology. New York and London, McGraw-Hill.
- SMITHBURN, K. C. & HADDOW, A. J. (1946). Isolation of yellow fever virus from African mosquitoes.—Amer. J. trop. Med., **26**, pp. 261–271.
- WILLIAMS, C. B. (1937). The use of logarithms in the interpretation of certain entomological problems.—Ann. appl. Biol., **24**, pp. 404–414.
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THE IDENTITY OF THE SPECIES OF *HYPODERMA* (DIPT.) ATTACKING GOAT.

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In northern and western Europe, the goat is not subject to flies that give rise to warbles apart from occasional attacks by *Hypoderma bovis* (L.) and *H. lineatum* (Vill.). The nasal and frontal sinuses, etc., of the goat are sometimes infested by *Oestrus ovis* L., but this does not give rise to warbles. Several species of *Hypoderma*, however, the normal hosts of which belong to the genus *Capra*, have been described from Mediterranean and Oriental countries.

The first species was described from the larva only by Brauer (1863) from a Bezoar goat (*Capra hircus aegagrus*) from Crete in the Zoological Gardens of Vienna at Schönbrunn. Many years later, Patton (1922) described the larva and adult of another species parasitising goats in the Punjab, and Austen (1931) those of a species found on goats in Cyprus. Brauer did not give a name to his larva (*l.c.*, pp. 134-135), but, in the index on p. 281, he referred to his description under the name of *Hypoderma aegagri*, this name being printed in heavy type and thus being included in his valid ("beibehaltenen") names. The following names thus refer to the regular parasites of goats:—

<i>Hypoderma aegagri</i> Brauer, 1863	Crete
<i>Hypoderma crossi</i> Patton, 1922	Punjab
<i>Hypoderma aeratum</i> Austen, 1931	Cyprus

These three species infesting goats have the mesonotum evenly sculptured, without four glossy black longitudinal weals, as in the case of *H. silenus* Brauer, which is believed to parasitise the donkey and perhaps the horse, and *H. cornuae* Crivelli (from *Gazella dorcas* L.), but unlike *H. bovis* (L.) and the majority of the species in the genus that attack cattle and deer.

It has been suggested by Henry (1931) that *crossi* and *aegagri* are the same species, since both attack goats. A comparison, however, of Patton's somewhat inadequate fig. 3 (the legend of which has inadvertently been placed under fig. 2 and *vice versa*) with Brauer's Plate viii, fig. 9, and description, shows clearly that the two species must be distinct. A study of the larvae of *H. crossi* (also figured by Soni in 1939) in the British Museum (Natural History) confirms this, although the discrepancy is not as great as the figures referred to above would indicate. The main differences between the actual larvae of *crossi* and *aeratum* are that the posterior dorsal rows of spines are absent from the larvae of *crossi* or indicated by only 1-2 odd spinules on one or two segments, whilst in *aegagri* and *aeratum* they are as a rule long and continuous and consist of numerous spines on at least 3-5 segments. Further, on the ventral surface the anterior belt of spines is either absent from the eighth abdominal segment of *crossi* or much reduced, while it is well developed in the Palaearctic species; the outer margin of the hind spiracles is, also, more crenate and less straight in *crossi*, the transverse rows consisting of 4-6 holes each, whilst in *aegagri* and *aeratum* they consist of 6-8. Obviously, zoogeographical considerations also would suggest some caution in synonymising *crossi* with *aegagri* or *aeratum*.

On the other hand, the fact that both *aegagri* and *aeratum* occur in the western Mediterranean would support their reduction to one species rather than the reverse. Austen described the larva of *aeratum* and his material is preserved in the British

Museum (Natural History). His fig. 3a certainly appears to be very similar to Brauer's fig. 9 of *aegagri*, the spinules showing almost exactly the same distribution and the hind spiracles being of the same shape. Austen, however, states that *aeratum* is distinguishable by the absence of small, smooth, button-like warts that occur on the dorsal surface and on the lateral callosities of *aegagri*. According to Brauer, one of these warts, each of which carries a small pit, is present on each lateral callosity and a pair on each dorsal and ventral fold of the second to tenth segments, but they are much smaller in *aegagri* than in *H. diana* Brauer. The dorsal rows of spines near the hind margins of the segments are restricted in *aegagri* to the posterior two thoracic and the first two abdominal segments, whilst in *aeratum* Austen described them as present on the posterior two thoracic and first four abdominal segments. Lastly, the anterior belt of spines on the median area of the last spinigerous segments are said by Austen to consist of a single row in *aegagri* and 2-3 in *aeratum*, but he obviously referred to Brauer's figure only, as the text described "meist zwei" rows for the dorsal and "zwei bis drei" for the ventral surface.

The first character, the pitted warts, is not shown in Brauer's figure of *aegagri*, but it is conspicuous in his figure of *diana* and this would seem to indicate that the warts were not easily seen in Brauer's larva. A typical specimen, for which I am very grateful to Dr. Max Beier of the Vienna Museum, confirms this fact. The warts are very inconspicuous, dull and shagreened like the rest of the integument, but each carries a minute whitish pit, probably the orifice of a gland. They are actually traceable on Austen's species, especially on the anterior segments, though they are less distinct and smooth than Brauer's description would seem to imply. The material that I have studied suggests that the warts are fully developed, button-shaped, and smooth only in the species with glossy weals on the thorax of the adults but vestigial and shagreened in the group of species which parasitise goats. There remains the difference in the number of segments which carry rows of small spines along the hind margin. In *H. aeratum*, these spines are considerably smaller on the second and third abdominal segments though still quite distinct even under an 8 \times hand lens. They show, moreover, some individual variation, but in the material at hand this is more conspicuous anteriorly than posteriorly, the row on the mesonotum being often almost absent. The typical specimen of *H. aegagri* received from Vienna has no posterior rows at all although it is undoubtedly of the third instar (being 18.5 mm. long and 8.5 mm. wide), but there is a single small spine present on the metathorax, first abdominal segment and third abdominal segment, the latter being the last segment but one furnished with a row of spines in *aeratum*. The type of *aegagri*, on the other hand, has well-developed rows on three segments and, according to supplementary information, kindly supplied by Dr. Max Beier, rows of very small spines occur on the next two segments.

The only remaining difference between the larvae of *H. aegagri* and *aeratum* becomes thus so slight and appears subject to so much variation on specimens obtained from one goat, that the two forms are certainly identical.

It is certain that *H. corinnae* has nothing to do with this group of species, as the anterior rows of spines are uniserial, the spines being very large and flat, forming oblong and ovate scales with truncate or rounded apices rather than spine-like asperities. The ovate shape of the body and the strongly developed scale-like spines of the prothorax, also, are very distinct from the corresponding characters of the goat warble flies. Two specimens collected by H. A. MacMichael at Gebel Sungur, N. W. Kordofan, 20.x.10, under the skin of a male dorcas gazelle, were determined by Gedoelst as *H. corinnae*. These specimens are in the British Museum (Natural History) and fit Crivelli's description perfectly. The larva was re-described and figured by Séguy (1933).

It can thus be said that the *Hypoderma* larvae from goats differ from those of the *bovis* group (*bovis*, *lineatum* and *diana*) in the weakly developed paired warts and that they are very different from *corinnæ* in the nature of the spines in the anterior row of each segment. Of the three names, *crossi* and *aegagri* unquestionably refer to two clearly distinguished larvae, whilst *aceratum* is identical with *aegagri*.



Facial views of the heads of *Hypoderma*.

Fig. 1.—*H. aceratum*, type ♂. Fig. 2.—*H. crossi*, ♂. Fig. 3.—*H. silenus* ♂.

(All figures made with camera lucida and Leitz microscope eye-piece 0 and objective 2.)

It is unfortunately not possible to compare the larval characters of *silenus* Brauer with those of the species discussed above, as the larvae have not been described and are not available.

With regard to the adults of the three species associated with goats, those of *aegagri* are unknown but those of *aceratum* and *crossi* belong undoubtedly to different species. Austen pointed out (*l.c.*, pp. 425-426) that in *aceratum* the tibiae are less infusate and the frons, and especially the interfrontalia of the male, much wider.

As the latter character has been somewhat discredited in other Calypttrata, a figure of the face of the two species is here reproduced, showing that they differ considerably in the shape of the clypeus and in the width of the facial keel. In *aceratum* (and therefore *aegagri*) the facial keel between the antennae is testaceous

and not, or not much, wider at the narrowest part than the base of the arista (fig. 1), whilst in *crossi* (fig. 2) it is fuscous on more than the ventral half and about three times the width. In *silenus* (fig. 3) it is even somewhat wider than in *crossi*, flattened on top and pale testaceous throughout. The adults thus prove that the Mediterranean goat warble fly is distinct from the Indian species and from *silenus*. Further distinguishing characters will be found in Austen (1931).

Summary.

The status of the species of *Hypoderma* that attack goats, and related species, is discussed. The Oriental species *H. crossi*, and the Mediterranean species, *H. acratum*, are shown to be distinct. *H. acratum* is known in the larval stage only, but as this is identical with that of *H. acratum*, the latter becomes its synonym.

References.

- AUSTEN, E. E. (1931). Bull. ent. Res., **22**, p. 423.
BRAUER, F. (1863). Monographie der Oestriden. pp. 134, 281.
HENRY, P. (1931). Bull. Acad. vét. France, (N.S.) **4**, p. 410.
PATTON, W. S. (1922). Indian J. med. Res., **10**, p. 574, pls. 30 & 31.
SÉGUY, E. (1933). Bull. Mus. Hist. nat. Paris, (2) **5**, p. 124.
SONI, B. N. (1939). Indian J. vet. Sci., **9**, p. 367.
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STUDIES ON THE SWARMING HABITS OF MOSQUITOS AND OTHER NEMATOCERA.

By Erik Tetens NIELSEN and Hans GREVE.

(PLATES VII, VIII & IX.)

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The researches described in the present paper were directed to explaining as far as might be possible certain remarkable phenomena associated with the occurrence of very large mosquito swarms near Pilehuset, a private laboratory in Northern Denmark. Work was carried on here from 1938-1940 and from 1945-1948; during the intervening period it was rendered impossible by the German occupation of Denmark.

Description of Study Area.

Pilehuset lies about 40 miles north-west of Copenhagen and some three miles from the sea, close to the north shore of Lake Arreso. During the Litorina period, the entire area was submerged but subsequent elevation produced open moorlands which, from the 16th century onwards, were buried under drifting sand. This process was arrested at the end of the 18th century by shore-line afforestation. The sandy swamps which resulted acquired a flora of *Erica*, *Calluna*, *Phragmites*, *Myrica gale* and *Salix repens* and peat-digging led to the formation of small, scattered ponds. This flora has yielded to a Birch-Willow-Alder association in recent years and an Oak-Rowan association is growing up. A small part of the area is under cultivation. The total extent of this part of the old inlet bottom is about 250 acres of which some 12 acres belong to the laboratory. Almost all the observations described were made in this latter area (Pl. VII). As shown in the photograph, the study area is bounded to the west by Road 8, to the south by Road 15, to the east by Road 20 and to the north by Ditch 25. The whole of this area is the property of the laboratory except for the two plots in the south-eastern and south-western corners. From Point 2, a small road (Valdemarsvej) runs eastwards past the entrance to the laboratory (23). To the north of this there is first a cultivated patch (1) and then, further east, an open area of grass and heather where many of the experiments with balloons and kites were carried out. This is bounded on the east by a peat ditch (21) and further east still lie old peat ditches, partly filled and overgrown by willows and reeds (19), and, finally, an area of heather with scattered birch trees (18). The observation mast (24), Stevenson screen (4) and rain gauge were placed in the corner of another patch of heather (5). The western end of this patch was used as a vegetable garden until 1943. There was a temporary pool surrounded by alder, willow and birch trees and bushes in the corner between the Valdemarsvej

A

and the drive near the observation mast. The projector (7) was placed at the southern end of the parking place by the laboratory near a small open space with grass and some birches (10). There was a garden round the laboratory (6) with poplar, white poplar and willow trees up to 70 or 80 feet high and to the north and east of this lay a meadow. The remainder of the area consisted of natural forest, mostly birch, with small patches of heather and bogs and many temporary pools (12, 16). Part of this area was crossed by a path which is now closed (13). A few observations were made in the area south of the laboratory property which consisted of a grassy plain, partly cultivated (9), a peat ditch (11) and a good sized reed-swamp (14).

Technique.

Time.—Times are given in M.E.T. and are one hour ahead of Greenwich Mean Time. They may be taken as accurate to within one minute.

Larvascope.—Observations on the numbers of larvae and pupae occurring in the temporary pools were made with a larvascope consisting of a plate painted white on the upper surface and fastened at right angles to the end of a stick. A suitable size of plate was found to be 6 ins. \times 6 ins. and when this was lowered into a pool it was quite easy to count the larvae even though the water might be so shallow that it was virtually impossible to catch them with a net. Larvae of *Chaoborus* and of CHIRONOMIDAE were studied during the first part of the investigation only. For this purpose we used a bottom sampler lent by Mr. C. V. Otterström to whom we are much indebted for his kindness.

Methods of observing swarms.—Much valuable information was obtained in the course of a daily walk round the laboratory with a notebook. Something new was almost always observed and after seven years' work we were convinced that no method of approach could compare in value with that of direct observation. A field glass proved most useful, and a monocular (8 \times 40) which could be carried in the pocket and a binocular (12 \times 50) were also used. Various kinds of lamps were employed at night, including one that could be attached to the forehead leaving the hands free. A motor boat head-lamp was used before the war with a 12-inch reflector and a 6 volt, 60 watt bulb which, when connected to the lighting circuit of an automobile, gave good service, but after the war this was replaced by a 20-inch Badger aerial projector (Pl. VIII c). This dated from the first World War but had been used by the Germans during the second on a Danish airfield. An arc-lamp with a consumption of about 5 amps provided the light-source. Telescopes (40–50 \times) were also used, one of which, an astronomical reflector with a mirror about 20 cm. in diameter, was kindly lent by the H. Struers Kemiske Laboratorium. The other, from an old theodolite, unfortunately gave a very small, dark picture.

Photographic Technique.—Attempts to photograph the swarms met with little success as, when seen close to, they are much more diffuse than might be supposed from the impression gained at a distance. It was easy to get pictures of the mosquitos, using a Ross Telecentric objective ($f=13$ ins.; 1 : 5.4), but they were too scattered for more than a few to be photographed together, even on a 13 \times 18 cm. plate. Illumination was obtained by using two flash bulbs mounted at the end of a long pole which was run up on a 30 ft. tower. This apparatus was positioned by means of strings attached to the end of the pole. Most of the other pictures were taken with an Ica Reflex (Ross Tessar 9 ins.; 1 : 4.5) or a Reflex Korelle (Tessar; 6 \times 9 cm. 1 : 2.8).

Catching Technique.—Free swarms out of reach of a net, but still fairly low, can be "called down" by singing in a certain interval about



Higher swarms, later in the evening, cannot be "called down" and for these a balloon net was used (Pl. VIII *a, b*). A detailed description is given of this method as it is not thought to have been employed before. The best type of balloon was found to be that used for carrying radio sondes. This is about 1 m. in diameter and can carry about 500–600 gm. The weight of the net used was 125 gm.; it was made of tulle and was 250 cm. long and 60 cm. across the mouth which was stretched on a light bamboo ring. The net tapered from before backwards for 100 cm. at which point it was 20 cm. in diameter; behind this the sides were parallel. The rear end was open and was closed when in use by knotting. The buoyancy of the balloon, whilst filling with hydrogen, was measured by means of a simple dynamometer made for the purpose. When filled, the mouthpiece was tied with a string about 8 m. in length, the other end being attached to a small ring holding the three bearing strings of the net. Another string was attached to the mouth of the net at the same point as one of the bearing strings and this was used for hauling down the net and the balloon. The altitude which can be reached with such a net depends on the weight of the string which must therefore be as light as possible. It must, however, also be strong since the winds encountered may be more violent than would be expected from observations made at ground level. An 0.8 mm. cotton string was used at first but later an angler's line. The line was wound on a 45 cm. wooden drum mounted on

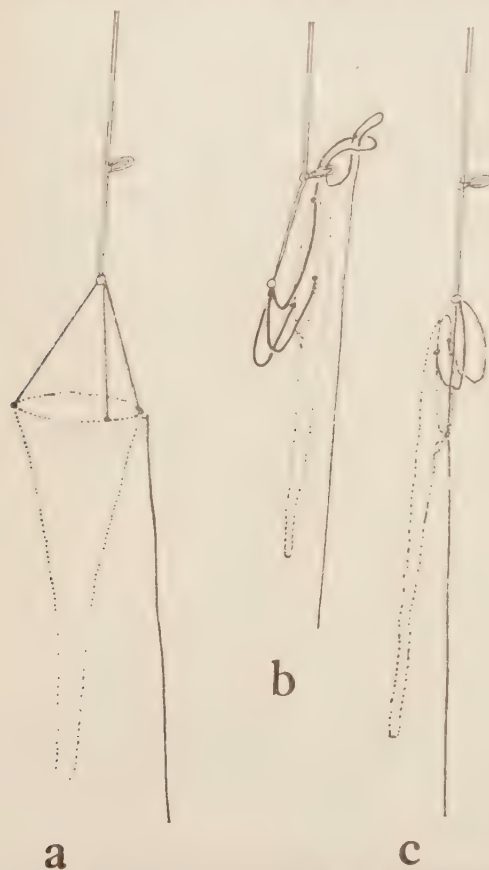


Fig. 1.—The Balloon Net. (a) Net open for catching. (b) Net ascending closed. (c) Net coming down closed.

wheels and fitted with a brake made from rubber tubing. When drawn down by means of the line the mouth of the net was pulled into a vertical position so that it was automatically closed (fig. 1 c). A fixed noose was made in the 8 m. cord which anchored it to the balloon before raising the net. This noose was made about 50 cm. from the point of attachment of the three bearing strings (fig. 1 b). Through it the line was drawn until it took the weight from the bearing strings (fig. 1 b). At this stage, the mouth was vertical and the net closed. The line was then fastened to the noose by means of a slip knot so that it could be detached by means of a gentle tug. A length of line equal to the height to be reached was unrolled from the drum and coiled loosely, and the balloon was released. When it reached the desired height, the line was detached from the noose and the net fell down into the normal position with the mouth open (fig. 1 a). Catching was effected by releasing the brake from the drum and allowing the net to ascend at full speed (about 200 m. min. or 12 km./hr.). Since the diameter of the drum was known, the length of line released could be roughly calculated by counting the number of revolutions. Owing to drift the height of the balloon, even in calm weather, was considerably less than the length of line released (about 230 m. for 300 m. of line, 260 m. for 400 m. and 280 m. for 500–600 m.) Three hundred metres could only be reached in exceptionally calm weather. The height of the balloon was calculated by estimating the angle between it and the ground ($h=1 \sin \alpha$). The error introduced by the fact that the line, "1", was curved was found to be negligible. The net was closed by means of a knot just behind the mouth in order to secure the contents for inspection. The knot at the bottom was then opened and a killing bottle introduced. Manipulations such as these were found to require practise by daylight before they could be carried out at night. When the weather was windy we tried using a kite or hauling the net to the top of the observation mast, but these methods were unsuccessful. Catching by means of a net stuck out from the cabin of an aeroplane was also tried (p. 242).

Listening Devices.—Attempts to locate the swarms by means of the sound which they emitted were not very successful. The humming tone was estimated first by blowing over the mouth of a bottle filled to various depths with water and later with an organ pipe equipped with a movable piston. A simple microphone with a trumpet 25 cm. in diameter and 200 cm. was used at first to amplify the sound made by the swarms. It was possible to hear the sound of a swarm with suitable amplification but it was usually drowned by extraneous noises. Later, the Danish State Broadcasting System lent a directed microphone and octave filter and this proved much more satisfactory although the results which it gave were still only a slight improvement on those obtained by unaided listening. We are much indebted to the Broadcasting System technicians and especially to Mr. Heegaard and Mr. Laursen for their help and advice in this connection.

Meteorological and microclimatic technique.

For temperature and humidity measurements, we used copper-constantan thermocouples with a separate copper cable for each and a single main constantan cable having a short branch to each junction. A double wire was used for the branch cables of 24-core 0.02 mm. copper and 42-core 0.015 constantan made specially for thermoelectric work by Nordisk Kabel-og Traadfabriker of Copenhagen and, for the main cables, a solid 1 mm. constantan wire with cotton insulation and a Siemens "Lackpapierdraht" 0.8 mm. copper one covered with a highly efficient protective varnish, two layers of waxed paper and two of silk thread, and an outer layer of wax. These cables lay in the earth for 11 years without giving any trouble and throughout this period the whole installation proved remarkably trouble-free. The inner junction was contained in a thermostat together with a thermometer. The Lange mirror galvanometer was provided with a temperature scale and used with full sensitivity but with a different resistance for each outer junction so that all readings could be

made directly in degrees centigrade. This meant devoting some time to the initial adjustments but proved to be well worth while in the long run (fig. 2). When making an observation, it was only necessary to adjust the light spot to zero and switch in the requisite outer junction. A green lamp was wired in parallel with the galvanometer lamp and the lamp used for illuminating the thermometer and a red lamp of higher wattage in parallel with the outer junctions. The former served to give warning

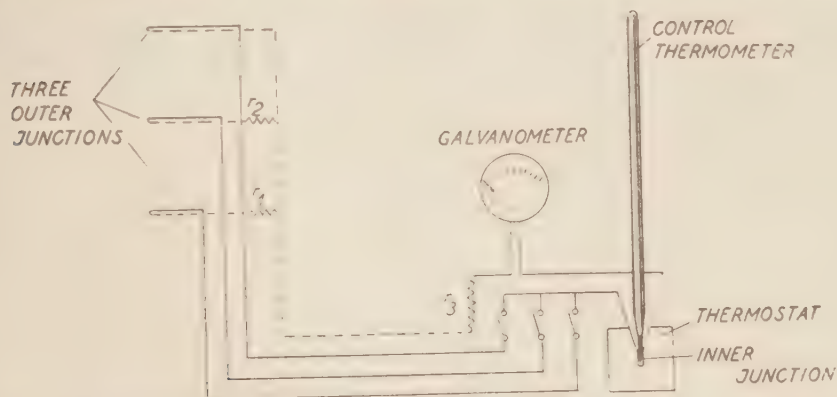


Fig. 2.—The Galvanometer Circuit. Copper wires shown by unbroken, constantan by broken line. r_1, r_2 resistances for balancing lead-in cables of different lengths. r_3 resistance for matching to galvanometer scale.

that the installation was switched on while the latter only glowed brightly when an outer junction was switched in and precluded readings being taken from two outer junctions simultaneously (fig. 3). With this installation more than 40 observation points could be wired in from the field although in practice not more than 18 were used at any one time. Attempts were made on several occasions to measure humidity by setting up a dry and a wet junction in close proximity but this method was not

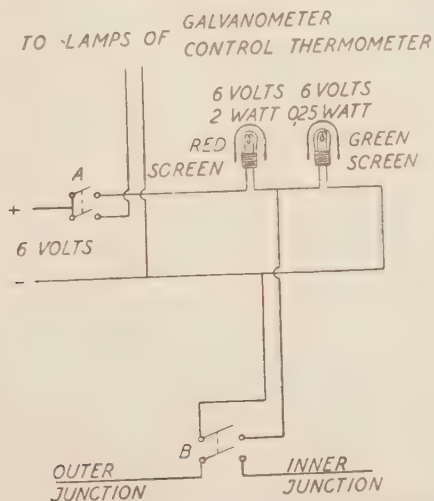


Fig. 3.—Circuit for warning lamps. When A is switched in, both lamps receive current but only the green one glows brightly enough to be seen. When B is switched in, the green lamp is short-circuited and only the red lamp glows.

found to be sufficiently reliable. Continuous temperature records were obtained with thermographs. One of these was placed in a Stevenson screen with an electrical thermometer near the sensitive part for control. Humidity was finally measured with an Assmann aspiration psychrometer kindly lent to us by the Nordisk Insulin-laboratorium. This was set up 2 m. above the ground, generally on the parking place near the projector.

Wind duration was ascertained by means of a vane on top of the observation mast 21 m. above the ground. Wind speed was measured by means of an anemometer, also on top of the mast and recorded by means of an electrically controlled counter in the laboratory. This apparatus, however, gave considerable trouble and it was eventually abandoned. Subsequently, visual observation of the effect of wind on trees etc., was relied upon.

Observations of cloud were made solely with the naked eye.

Rainfall was measured by means of an ordinary rain gauge placed between the mast and the Stevenson screen.

A simple sunshine recorder was operated as part of the normal laboratory routine.

Regular observations of light intensity were not started until 1946. That year we used a simple comparometer, in which the light from an electric lamp was passed through a semi-transparent screen half covered by a white plate, to reflect light from the surroundings. The voltage was kept constant by means of a variable resistance in series with it. The intensity of light which it emitted was adjusted by a second resistance, also in series, until it was equal to that reflected from the white plate. A red filter of glass or cellophane (fig. 4) was used to eliminate the difference in colour from the two sources. Results from this were encouraging and in 1947 we went on to use a photoelectric cell which was fixed 10 ft. above the ground in the parking place and wired to the Lange galvanometer. This exhibited some changes in sensitivity, especially after wet weather and the last few observations in 1948 were made by

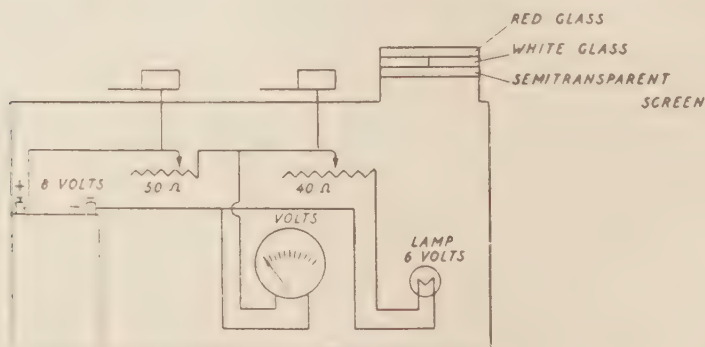


Fig. 4.—Circuit of comparometer for light intensity measurements.

means of a technique similar to that employed during 1946. This was also adopted during the experiments in the constant temperature room described below. We are much indebted to Mr. Tage Larsen for the loan of a lamp from the biophysical laboratory of the University of Copenhagen which we used for testing our comparometer and to Professor Georg Weber for having our two later instruments tested in the electrotechnical laboratory of the Technical High School. The light intensities given should not be taken as exact but they are considered to be of value since they are comparable with one another, having been made with the same instrument.

Ethology.

Larval bionomics.—The thorough studies of Wesenberg-Lund (1921) rendered a detailed investigation of larval bionomics unnecessary but it was found useful to study larval densities in order to predict when the first swarms might be expected and when they might be considered to be at their maximum, *i.e.*, when the last pupae disappeared from the pools. The two commonest species, *Aedes communis* (Deg.) and *A. cantans* (Mg.), were found as larvae in the same ponds and they were found to behave in very much the same way except with regard to the times of their first appearance (see below). Some of the figures obtained with the larvascope (p. 228) (each the average of several counts) are given in Table I. The increase in numbers which took place up to about 15th May is thought to have been due to concentration resulting from the drying up of the pools; after that date, this effect was probably

TABLE I.
Larvascope Counts.

Locality	Plate I	Date (1946)															
		8/5		9/5		12/5		14/5		17/5		20/5		23/5		29/5	
		L	P	L	P	L	P	L	P	L	P	L	P	L	P	L	P
Mulehul	23	26	0	30	0	22	0	45	5	50	0	50	23	15	75	10	55
Valdemarsvej	22	22	0	30	0	25	2	20	10	20	5	15	5	30	20	25	25
Menyantheslul	17	50	2	60	0	50	5	30	10	40	2	15	10	20	5	25	15
Grænsegroft	16	10	0	20	0	20	5	25	5	10	15	10	15	2	30	5	2
Grøft ved nedlagte Sti ...	13	60	10	60	10	40	15	60	20	40	0	50	5	—	—	—	—
Sverfilds Dam	15	13	5	20	0	20	15	40	15	10	15	15	5	20	2	10	10
Gravhul	12	3	0	10	0	20	1	15	5	10	0	0	5	0	5	0	3
Total		184	17	230	10	197	33	246	70	150	37	155	68	78	137	75	110
Total		201		240		230		316		187		223		224		185	
$\frac{P}{100 \frac{P}{L+P}}$		8		4		14		22		20		30		61		60	

L. Larvae. P. Pupae.

offset by the emergence of adults. The first swarms were observed on 12th May in the year in question. Larvae of Nematocera other than CULICINAE were studied during the early part of the investigation. Those of *Chaoborus crystallinus* (Deg.) were found in the peat ditches and in the nearby Lake Arreso. As a rule, the first and last adults came from the peat ditches but the main swarms in July emanated from Lake Arreso. Chironomid larvae were found in the same breeding places, the commonest being a *Chironomus* of the *plumosus* group which bred at the bottom of Lake Arreso.

Phenology and Longevity.

The difference in time of first appearance of *A. communis* and *A. cantans* observed by Wesenberg-Lund (1921) was noted by us during every year of our investigation, the latter species appearing a week or so after the former. In 1948, the first swarms were observed on 30th April and, in 1939 and 1941, on 23rd and 24th May. In other years the dates fell between these extremes; 11th May in 1945, 12th May in 1947,

15th May in 1940 and 16th May in 1946. In every case the swarms made their first appearance two or three days after the dominant tree in the study area, the birch, came into leaf. As far as we could see, the length of the swarming period depended on the size of the mosquito population and the type of weather experienced. The population size is in part the result of climatic factors, being dependent in particular on the degree of inundation of the countryside. The eggs of the species in question are laid dry and will hatch only when flooded. When prolonged frost is followed by heavy downpours, very many eggs will be hatched and large numbers of larvae will follow the water down into the temporary pools in which it accumulates. Years in which the ground is only lightly frozen so that the surface water is soon absorbed, or in which the rainfall in winter and early spring is light, will be characterised by a small mosquito population. The first swarms appear a few days after the first emergence of mosquitos. Males and females seem to make their first appearance at the same time and, as noted by Wesenberg-Lund, the females are very long-lived, some surviving into August. Little is known concerning the longevity of males. The size of the adult mosquito population increases rapidly during the first fortnight of the swarming period and remains fairly constant until about ten days after the pools dry up and the last adult emerges. For this reason, we believed the length of life of the male to be about a week or ten days, but this impression requires confirmation. Our observations revealed the fact that in years in which the mosquito population is large the swarming season is longer than in those in which the population is small. This appears to be due to the fact that in years in which the mosquito density is large the minimum number required for swarm formation is reached during an earlier part of the breeding period than when it is small and the swarming season is accordingly longer. Climatic factors also appear to play a part, cold weather delaying the emergence of adults and so prolonging the swarming period.

Diurnal activity of adult mosquitos.

The following description applies to *Aedes cantans* but, according to our observations, it also holds good for *A. communis* and for other species such as *A. punctor* (Kirby) and *Theobaldia morsitans* (Theo.), which were encountered from time to time. The period of full daylight is spent in the shelter of the grass and bushes and under trees. At this time the mosquitos rest quietly, except for a slow movement of one of the hind legs which is suggestive of the presence of a sense organ or organs in this region. Occasionally, a short flight is made varying from a few inches to two or three yards. The great majority of resting mosquitos are difficult or impossible to detect and the proportion in flight at any one time appears larger than it really is. Actually it is very small. A few are, however, always to be seen in flight, most of them females. As the shadows move across the grass in the course of the day, the mosquitos follow them in this way and the short flights referred to always end in a shady place. The males tend to rest in the deeper parts of the vegetation and it is necessary to sweep with a net in order to get an accurate estimate of the proportion of the sexes. Both sexes rise in the air in great numbers when disturbed, the males to escape, the females often in search of a blood-meal. In order to observe the behaviour of undisturbed mosquitos it is necessary to lie still for a long time and even then some females are likely to come in quest of a blood-meal. In our experience, the best observations were obtained by the use of a powerful field glass or telescope (p. 228) at a distance of eight or ten yards.

Feeding habits.

Most authors agree that the blood-meal serves only to provide the mosquito with additional food substances needed for egg-production, probably proteins. *T. morsitans*, like a number of other species, has never been observed to take a blood-meal and this may be related to the fact that it has a much shorter life than the species of *Aedes* which we encountered in the course of this investigation. Although this was

outside the scope of the main investigation, we devoted some time to the study of feeding habits since, apart from a few isolated investigations, little is known concerning the ordinary food of the adult mosquito. Wesenberg-Lund (1921) noted that Culicids may be found with the abdomen swollen with a clear, colourless, sweet-tasting fluid and a number of instances have been cited by Howard, Dyar and Knab (1915) and Marshall (1938) of mosquitos feeding on such substances as milk, nectar, port wine and plant juices. We ourselves have seen them feed on sweet cake and on treacle used for trapping moths. In 1939, Dr. M. Weitze of the Nordisk Insulin Laboratorium kindly made a micro-determination of the crop contents of four *A. cantans* (two of each sex) with the abdomen swollen as described and found a total sugar content corresponding to 0.4 mg. glucose, an average of 0.1 mg. for each individual. We investigated the time of feeding by catching mosquitos at intervals during the day and counting the numbers with markedly swollen abdomens. The abdomen remains swollen for some hours after the meal. The results of two typical sets of observations, one made in 1939 and one in 1948, are given in Table II. The first set of figures has been based on observations by the senior and the second on observations by the junior author in order to minimise the personal element. Both give the same general picture of two meals during the course of the day, one just after the descent from the morning swarms and the other just before the ascent in the afternoon. Observations published during the preparation of this paper also indicate

TABLE II.
Observations on Feeding Times.

1st June 1939.

26th-27th May 1948.

Hour	Caught	Abdomen swollen		Hour	Caught	Abdomen swollen	
		Number	%			Number	%
03.15	5	0	0	12.00	236	6	3
03.30	4	0	0	13.00	176	4	2.3
04.00	27	0	0	14.00	95	6	6
04.35	42	1	2	15.30	97	4	4
05.00	16	4	25	16.00	81	2	2
06.00	196	154	78	17.00	137	20	15
11.00	94	52	55	18.00	167	111	66
14.30	98	22	22	19.00	160	142	89
17.30	61	48	78	22.00	22	20	90
				00.00	7	4	57
				02.00	29	8	27
				03.00	26	1	4
				04.00	196	34	35
				04.30	159	101	64
				05.00	234	191	82
				07.00	101	70	70
				08.00	97	33	33

a daily activity with peaks of feeding in morning and evening (Larsen, 1948). Our observations convinced us that the meals are in fact taken at these times but direct observation of feeding mosquitos proved to be unexpectedly difficult. The meal is taken very rapidly and the mosquitos may be seen drifting down in the morning (p. 243) with the abdomen completely collapsed while a few moments after landing among the vegetation it is so swollen as to be clearly visible through a field glass at a distance of ten yards. Observations with a hand lens carried out on mosquitos given sugar water in the laboratory showed that they avoided the larger drops and took only the very smallest. Most of the time spent in feeding seemed to be devoted to finding drops of a suitable size and the actual intake took only a few seconds. We were only twice successful in finding the actual food source, in one case a willow (*Salix pentandra*) of which there were only four or five bushes in the whole study area, and, in the other, a whitethorn (*Crataegus oxyacantha*) (Pl. IX). It was evident from a comparison of the numbers of mosquitos seen feeding on these plants and the total size of the population that neither the willow nor, still less, the whitethorn was the principal food source. The morning meal appears to be made in the grass and among the low vegetation and we tried feeding fluid expressed from chopped grasses and other herbs to mosquitos in the laboratory. They refused this but fed instantaneously when transferred to sugar water. Even sugar water was, however, refused when the concentration was below about 2 per cent., i.e., approximately the level at which the sweet taste ceases to be perceptible to human beings. In our opinion, the principal source of food appeared to be honey-dew produced by Aphids, enormous numbers of which were to be found on the birch trees. The lower vegetation is generally covered with this substance.

Mating.

Having regard to prevailing ideas on the subject, it might be thought more appropriate to include the present section in that part of the paper which deals with swarming. The investigations showed, however, that these activities normally take place at quite different times and the writers are convinced that the belief that the swarm is formed by males and that the females are attracted by the sound which these males emit and enter the swarm for the purpose of copulation is entirely erroneous. Mating may occasionally take place when both sexes are flying up in the evening, the males to swarm and the females to seek the shelter of the tree-tops (p. 242), but this is exceptional. Ten mosquitos per square foot of shaded ground during the daytime may be assumed as a moderate estimate. Since more than half the 12 acres of ground in the experimental area is shaded, this would give a total population of roughly three million mosquitos. This is, of course, only a very approximate figure but, in view of the fact that, on an average not more than two or three matings were observed to take place during each evening ascent, it will be realised that only a small percentage of all the matings can take place at this time. We only observed three copulations during the whole of the investigations amongst several millions of mosquitos actually taking part in the swarms, so that it is clear that the swarm itself cannot be regarded as subserving the mating function. Howard, Dyar and Knab (1915) noted two positions assumed during copulation, one with the two individuals flying end to end with their heads directed to opposite points of the compass and the other in which they are clasped face to face with the bodies vertical. It was at one time suggested that the attitudes were characteristic of different genera of mosquitos but Marshall (1938) observed that *Culex molestus* Forsk. in captivity at first come together in the vertical position and later spread out into the horizontal. We ourselves observed *Aedes cantans* many times in both positions and there is no reason to doubt that the process is the same in this species. Mating normally takes place either in the morning, after descent to the ground, in which case the couples fly together above the ground for only a short time, most of the brief copulation taking place among the vegetation, or in the afternoon when the whole copulation frequently takes place in the air.

Evening swarming.

The approach of man during the daytime often causes the mosquitos to rise from the grass thus producing the appearance of " swarms " of short duration. On these occasions, the males quickly return to the grass and the females follow them as soon as the chance of a blood-meal has passed. After about 17.00 hrs. these " provoked swarms ", as they may be called, are of longer duration and the males are the last to leave. Such swarms were observed until about 18.00 to 19.00 hrs. and it was not until after working for two seasons that it was realised that they were not spontaneous but provoked by the observer. It is believed that many swarms described by earlier workers have been of this type. Spontaneous swarming starts about one hour before sunset but the precise time varies from day to day and may be as early as 18.00 hrs. or as late as 20.45 hrs. These swarms, which comprise male mosquitos only, are of the nature of " free swarms " and they are not associated with any

TABLE III.
Duration of evening top-swarms.

Date	Duration	Date	Duration	Date	Duration
1939		1945		1947	
30/5	-21.30	25/5	20.06-	14/5	-20.46
31/5	20.25-21.32	28/5	-21.24	15/5	-20.47
2/6	-21.53	29/5	20.45-	16/5	-20.57
3/6	20.15-21.25	31/5	20.25-21.18	17/5	-21.01
4/6	-21.52	1/6	-21.30	18/5	20.22-20.32
5/6	-21.26	2/6	20.40-21.34	19/5	-20.57
6/6	-21.32	3/6	20.38-21.37	20/5	20.23-20.57
7/6	-21.58	5/6	-21.40	21/5	-20.58
11/6	-21.36	8/6	-21.38	22/5	19.55-21.15
12/6	-21.35	11/6	-21.44	23/5	19.43-21.25
1940		12/6	-21.45	24/5	19.15-
20/5	-20.39	1946		25/5	18.53-21.23
21/5	19.55-20.55	21/5	-21.07	26/5	18.35-
22/5	19.52-21.08	22/5	-21.16	27/5	19.56-21.36
23/5	19.57-21.17	23/5	-21.21	28/5	19.50-
24/5	-20.57	24/5	-21.09	29/5	19.43-21.30
25/5	19.35-21.18	25/5	-21.14	1/6	-21.52
26/5	19.40-21.30	26/5	19.52-21.15	3/6	20.18-21.20
27/5	19.55-21.18	27/5	19.50-21.28	5/6	20.28-
28/5	19.28-21.13	28/5	19.58-21.25	6/6	19.10-21.31
29/5	19.25-21.18	29/5	20.30-21.25	9/6	19.59-21.40
30/5	19.50-21.28	30/5	20.20-21.25	11/6	-21.52
31/5	19.40-21.23	31/5	20.23-21.23	12/6	20.35-21.37
1/6	19.40-21.33	1/6	20.24-	13/6	20.32-21.43
2/6	19.40-21.30	2/6	-21.35	14/6	20.38-21.38
3/6	20.03-21.43	3/6	20.30-21.34	15/6	20.39-21.40
4/6	19.45-21.29	4/6	20.24-21.40	16/6	-21.45
5/6	20.12-20.53	5/6	20.29-21.25	17/6	20.41-21.52
6/6	20.37-21.55	6/6	20.22-21.46	18/6	20.46-21.52
7/6	-21.52	7/6	20.18-21.39	19/6	-22.09
8/6	-21.56	8/6	20.15-21.26	20/6	20.38-21.49
9/6	20.48-21.59	9/6	20.17-21.32	1948	
10/6	-21.36	10/6	20.27-21.47	30/4	19.27-20.08
1941		11/6	20.27-21.46	1/5	19.45-20.17
23/5	20.39-20.50	12/6	20.12-21.45	6/5	19.23-20.38
24/5	20.15-20.51	13/6	20.30-21.43	7/5	20.00-20.45
1945		20/6	20.14-21.46	8/5	18.25-20.37
11/5	20.09-20.50	21/6	20.12-21.42	9/5	19.00-20.52
13/5	19.25-21.11	22/6	20.26-	10/5	18.53-20.52
20/5	20.00-21.13	1947		11/5	18.27-20.50
21/5	-21.10	12/5	-20.36	16/5	18.45-20.46
22/5	-20.58	13/5	-20.37	20/5	19.11-20.15

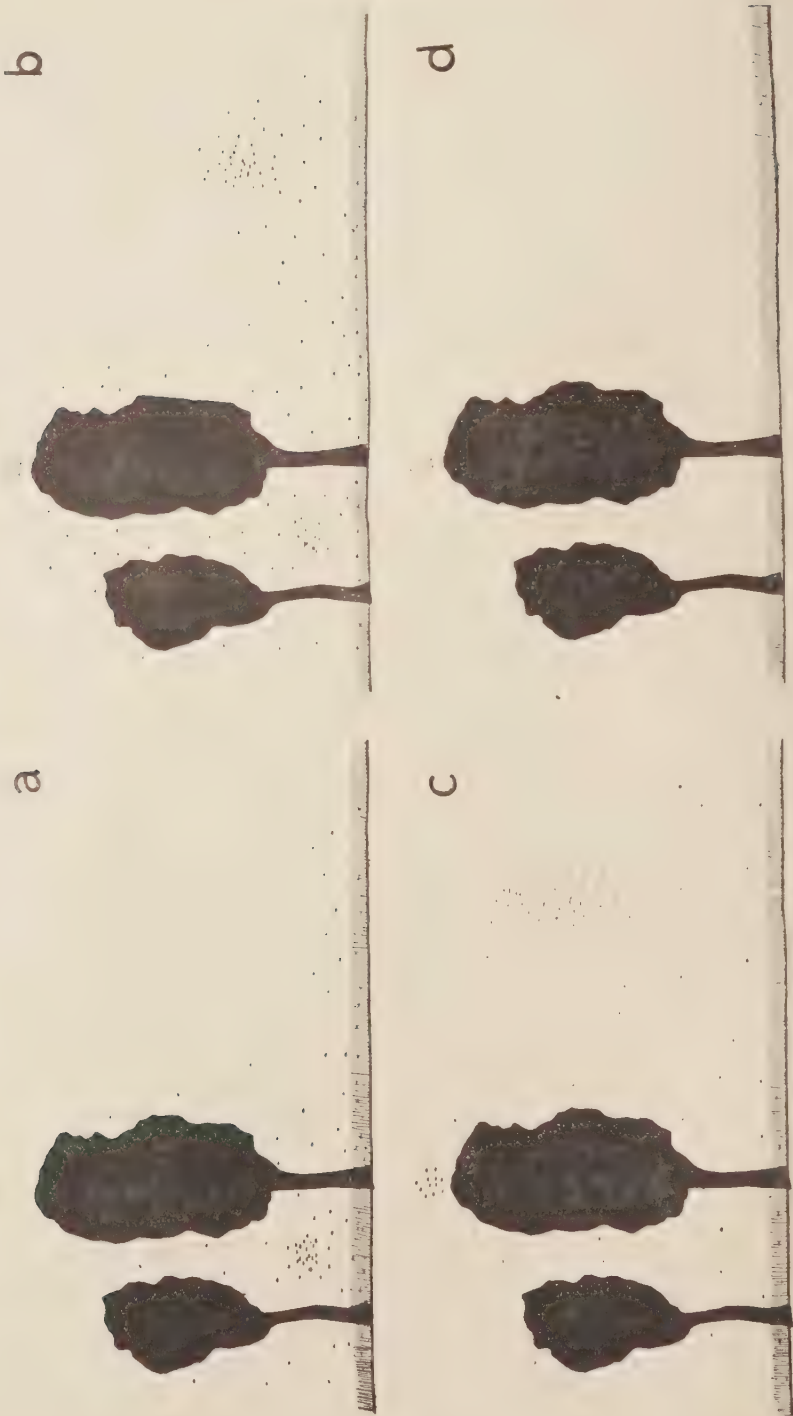


Fig. 5.—Phases of swarming and different types of swarm encountered. (a) The ascent, free ground swarms between the trees. (b) Later stage of ascent showing ground swarm, top-swarm and free swarm. (c) Disappearance of ground swarm. Free swarm higher in the air. (d) End of swarming. Top-swarm fading away.

particular object in the landscape. They were termed "ground swarms" in order to distinguish them from other types of free swarms because they occur only about 1 m. above the vegetation. They resemble the provoked swarms in appearance and are found most commonly in sheltered places among trees (fig. 5 a). It is thought that the many references in the literature to swarms observed in shafts of sunlight between the trees relate to this type of swarm. Simultaneously with these swarms, isolated individuals of both sexes may be seen at a height of 2-4 m. and copulation sometimes occurs. Shortly after this, the bulk of the mosquito population becomes involved in an upward movement from the ground which was termed the ascent. Those males which are not involved in ground swarms seek the nearest tree and follow the foliage to the top. Some females gather round the observer for a blood-meal but the majority follow the males to the tree-tops though more sporadically. Those individuals that have arrived at the tree-tops now form "top-swarms", each associated with a particular tree (fig. 5 b). We have occasionally seen such swarms formed over poles with wires but the great majority are found above trees. The top-swarms start a little later than the ground swarms, the precise time varying from day to day between about 18.15 and 20.55 hrs. In general, they begin about an hour before sunset during the early part of the swarming period and about half an hour before sunset during the later part. The top-swarms are generally small, rarely with more than 20 to 30 individuals. They often start simultaneously over all the tree-tops but sometimes they are formed sporadically in different parts of the area starting from only one or a small number of individuals. Cessation is gradual, the individuals disappearing one by one over a period of about a quarter of an hour, but the time of disappearance of the last individual is very constant, much more so than the time of commencement of swarming (fig. 6 and Table III). The curve does not follow that representing the time of sunset exactly but is a little steeper so that the cessation of swarming occurs later in relation to the time of sunset as the season wears on. This appears to be connected with the increased duration of twilight during the latter part of the season and its possible relation to light intensity is discussed below (p. 246). For the present, it is merely wished to emphasise the

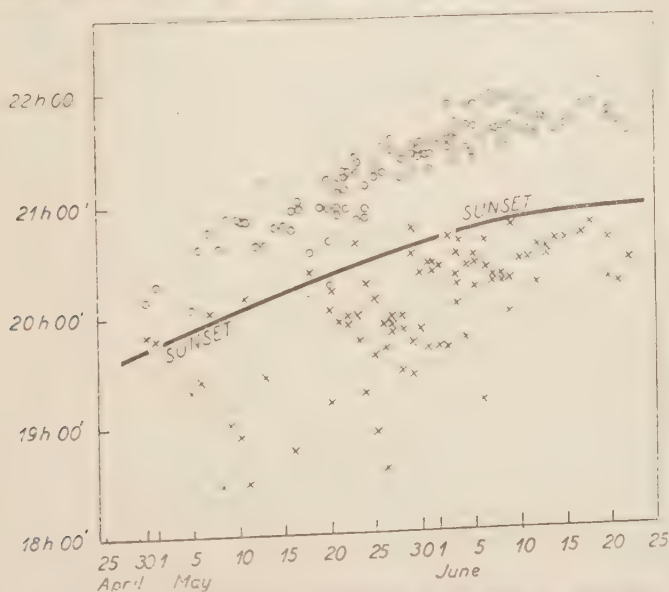


Fig. 6.—Times of beginning and end of top-swarms in the evening, 1939-48. Beginning of swarming indicated by a cross, end of swarming by a circle.

remarkable regularity with which the phenomena occurred. It was possible to predict the time of cessation of the top-swarms within about \pm ten minutes over a considerable period covered by the observations. Thus, on 3rd June the times concerned were 21.25 hrs. in 1939, 21.43 hrs. in 1940, 21.37 hrs. in 1945, 21.34 hrs. in 1946 and 21.20 hrs. in 1947. This held good despite the fact that the weather on the date in question was very varied, being calm and cloudy in 1939, very warm in 1940, slightly windy in 1945, rainy with a south-westerly gale of 5 Beaufort in 1946 and cool with a fresh breeze from the North-West in 1947. Other natural phenomena were also observed to take place with great regularity in relation to the swarming times, the evening flight of the swallows beginning at about the same time and ending about 20 minutes earlier, the evening concert of the green frogs starting just after sunset and the goat-sucker starting and the first bat appearing just after the last mosquito disappeared.

The number of individuals concerned in the top-swarm remains fairly constant during most of its existence but the swarm is not static. Individual mosquitos constantly leave and others take their place. On the 9th June, 1939, an opportunity to study this phenomenon in detail was provided by a swarm of 10 to 20 individuals that formed over the weather screen. We caught all the individuals in the swarm with a net at 20.58 hrs. and found that they comprised 14 ♂ *Aedes cantans*. Four and a half minutes later, there were again approximately 12 to 14 individuals in the swarm. It was calculated that, during its existence, some 200 to 400 mosquitos must have participated in this swarm, each occupying it for five to ten minutes at most. These mosquitos come either from the ground, ascending the trees in the manner described, or from the free swarms which are formed after the disappearance of the ground swarms.

It appears that all the male mosquitos in flight take part in either the top-swarms or in free swarms which are very large and have their base at first about 2 m. above the ground. Later, they move up to 4-5 m. and the top may be anything from 5-12 m. above the ground. Their formation appears to be independent of that of the ground-swarms. They do not occur in the same place and on occasion they begin to form before the ground-swarms have disappeared. The same types of swarms seemed to be formed in the same places year after year. The free swarms disappear gradually and finally cease shortly before the numbers of individuals in the top-swarms begin to diminish. The mosquitos dance above the tree-tops for five to ten minutes as they leave the swarms forming an "intermediate passage" swarm before descending to their night quarters in the trees. The diminution and finally the disappearance of the top-swarms follows shortly afterwards, taking about 10 to 20 minutes and this normally concludes the evening's swarming (fig. 5 d). Occasionally, however, a third type of free swarm is formed after the disappearance of the top-swarms. These swarms have their bases near the tree-tops 15-20 m. above the ground and extend up to a height of about 30 m. These are termed "ceiling swarms" because they tend to spread out and coalesce at the top giving the impression of a vast layer of mosquitos above the tree-tops. They last for about an hour after the top-swarms and fade away gradually at a time when it is too dark to see them without the projector. We observed them only three or four times in 1947 and once or twice in 1948.

Movement within the swarm.

Movements of individuals within the swarm are of the same kind whatever the type of swarm but their nature varies somewhat with the wind speed. The mosquito flies in a circle of 20-50 cm. diameter parallel with the ground with winds of 0-1 Beaufort. The direction of flight is normally reversed after each circuit, the mosquito pausing momentarily at the turning point with its head pointing into the wind. The turning point occurs where the circumference is intersected by a diameter in line with the wind direction (fig. 7 a). Occasionally, two circuits are made before changing direction (fig. 7 b) and at irregular intervals a short ascent or descent is made at the

turning point so that the plane of flight is elevated or depressed in a vertical direction. When the wind is weak and its direction varies from place to place, the direction of flight varies from swarm to swarm but in strong winds all the mosquitos in all the swarms turn opposite the same point of the compass. The circle is drawn out into an ellipse with winds greater than 1 Beaufort and, above about 3 Beaufort, it assumes the form of a straight line pointing in the direction of the wind, the mosquito reversing direction at each end. Movements within the swarm are more rapid in windy than in calm weather and general activity within the swarm appears to be higher on windy than on calm nights. Sometimes, when the swarm is broken up by a sudden gust of wind, the sound which it emits becomes louder and higher in pitch until it is reconstituted. The mosquitos may seek shelter in the lee of the tree-tops in high winds. If the tree-top is then bent down by a sudden gust, the swarm will be displaced but on several occasions we have seen the original position regained before the tree-top was back in place again.

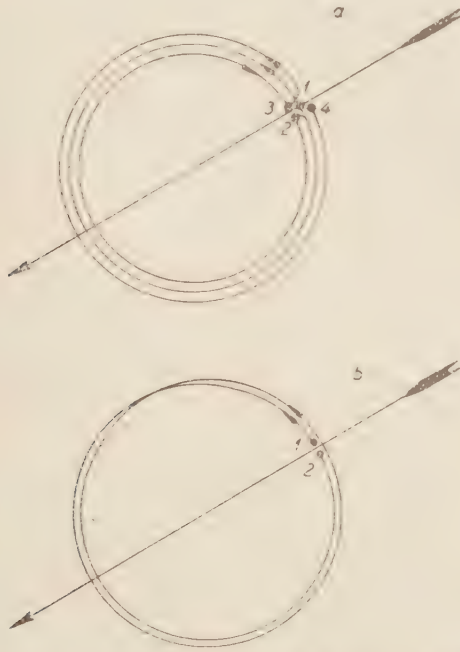


Fig. 7.—Movements of an individual mosquito in the swarm. The arrow indicates the direction of a very gentle wind. For explanation see Text.

Night-time resting places.

The most difficult problem was to ascertain the whereabouts of the mosquitos between the time of their disappearance from the evening swarms and their reappearance in the morning ones. Samples of 100 to 200 could be caught during the daytime with a few sweeps of the net but after 22.00 hrs. only very small numbers could be obtained, even by energetic sweeping over a wide area. The difference was very striking and it is remarkable that it should not have been referred to in the literature. During 1939 we inspected all the likely places including the tops of trees which were beaten with long sticks; to disturb the highest branches we used a bow and arrow and even a gun, employing the light beam from the projector to show up any mosquitos that emerged. Having failed to detect anything approaching the

numbers of mosquitos observed during the daytime or in the swarms, balloon catches were attempted in case the swarms had merely ascended higher in the air. The numbers taken were, however, still very small. In 1945 we resorted to the use of an aeroplane and Captain M. Hansen, R.A.F., made a daytime reconnaissance flight over the study area, and one of us (H. G.) made a night flight with Mr. Simonsen of "Zoneredningskorpset" who kindly provided a suitable plane. This flight lasted from 23.02 to 23.29 hrs. on 29th May and catching was carried out at all heights from 200-1,000 m. No mosquitos whatever were caught and it was concluded that they must be below 200 m. and that it ought to be possible to detect them by means of a suitable light source. The large projector already described (p. 228) was, therefore, obtained and used in conjunction with telescopes. Again, the results were entirely negative. Finally, in July, we tried sweeping the tree-tops with the balloon net. By this time there were few mosquitos to be found, even in the daytime, but we obtained many Chironomids. Using the same methods as in 1939, we could find no Chironomids but sweeping with a net immediately afterwards revealed them in large numbers. In 1947 we repeated the balloon net technique earlier in the season, and the same results with mosquitos were obtained. Accordingly, our final conclusion was that the latter do in fact rest in the tree-tops between the evening and morning swarms but that our methods adopted to detect them visually were inadequate. Apart from this, small numbers of mosquitos may be found near the ground during the night. We assumed that these were freshly hatched. The proportion of females near the ground became higher during the latter part of the season and we thought these were fertilised females.

Morning swarms.

These normally start 30 to 40 minutes before sunrise, but, exceptionally, they may start as early as 01.30 hrs. They were often detected first by the sound which they emitted. Free swarms were found in the same places as in the evening. The times of beginning and ending of swarming bore a similar relationship to the time of sunrise (Table IV and fig. 8) and, in general, the cycle of events was the same as in the evening

TABLE IV.
Duration of morning top-swarms.

Date	Duration	Date	Duration	Date	Duration
1939		1946		1947	
4/6	03.15-04.04	27/5	02.47-03.56	3/6	03.15-03.57
17/6	02.32-03.52	30/5	02.35-03.39	8/6	03.15-03.45
1940		1947		9/6	03.05-03.27
24/5	03.08-03.52	18/5	03.30-04.35	10/6	02.50-04.20
26/5	03.00-03.55	19/5	03.35-04.00	15/6	02.46-03.18
27/5	02.50-03.55	24/5	-05.04	16/6	02.58-03.39
28/5	03.09-03.57	26/5	-04.25	17/6	03.10-03.28
1/6	03.13-03.50	28/5	03.20-04.13	18/6	03.10-03.36
3/6	02.50-03.30	29/5	-04.13	19/6	03.12-03.45
1945		30/5	02.57-	1948	
20/5	03.27-04.15	31/5	03.00-05.08	7/5	03.52-04.24
29/5	03.20-04.15	1/6	-04.08	10/5	03.32-04.43
—	—	2/6	02.58-03.54	—	—

but in the reverse order. The various phases tended, however, to be less sharply defined and were often of shorter duration. Under certain conditions, to be described below, swarming does not take place and the mosquitos descend straight from the trees almost like falling snow, a most striking phenomenon. On these occasions, the entire descent takes about 10 to 15 minutes.

Swarming of *Chaoborus*.

The swarming habits of *Chaoborus crystallinus*, which breeds both in Lake Arresø and in the peat ditches, were similar to those observed in the case of the mosquitos. It succeeds *Aedes cantans* as the dominant species during the latter part of June. The tendency to form very large free swarms is more pronounced but, as this also characterises *A. cantans* under favourable conditions, it may well be due to the warmer weather. *C. crystallinus* is less confined to shady places during the daytime and may be found in the sunshine and on bushes and walls. We observed all the different types of swarms formed by this species, low free swarms (perhaps less often than in *cantans*), top-swarms, free swarms with a nucleus and, on one occasion a very large ceiling swarm. The latter was observed at about 20.45 hrs. on 7.vii. 1939, swarming

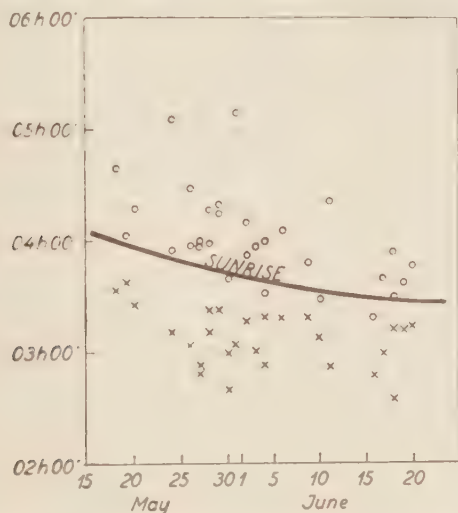


Fig. 8.—Times of beginning and end of top-swarms in the morning, 1939–48. Beginning of swarming indicated by a cross, end of swarming by a circle.

having begun about ten minutes before. On this occasion the sky was covered with light cloud and there was a S.E. breeze of Beaufort 2. The humidity during the afternoon had been unusually high. The entire heather area was covered by a vast ceiling swarm at about 20–30 m. with dependent columnar swarms continually pushing down towards the ground, engulfing one of the numerous small swarms occurring at about 0.5–2 m. and then retracting. The whole phenomenon was most impressive. It appeared probable that the swarm extended along the whole N.W. border of Lake Arresø. Downward movements of the columnar swarms ceased at about 21.15 and five minutes later the swarm disappeared.

The humming of the swarms can be heard just as dawn begins, at about 02.30 hrs., and they can be seen five or ten minutes later in the same places as in the evening. We did not see any top-swarms in the morning but these may well have occurred as only a few observations were made. Descent from the nucleated swarms took place in the reverse manner to that described above, a columnar protrusion being pushed down towards the ground, part of this breaking off and, a few seconds later, a small "rain" of *Chaoborus* falling to the ground. The very large *Chaoborus* swarms from Lake Arresø came into the area with S.E. winds and, as these do not commonly occur when this group is abundant, their appearance was very sporadic. It was only

in 1939 that any considerable number of large swarms were seen. Every June, however, we encountered a number of small swarms from the peat ditches mixing with the last *A. cantans*. Small larvae were found in the peat ditches in late July and early August following the swarming of the July brood from Lake Arresø. These give rise to a second, weaker generation observed by Meinert (1886). We did not see any swarms of these late *Chaoborus* but they are probably quite numerous.

Swarming of Chironomids.

Chironomid swarms consisting mostly of *Chironomus plumosus* (L.) came into the area with the S.E. winds from Lake Arresø. They were encountered during the latter part of May and June together with those of *A. cantans* and behaved much like this species. The top-swarms usually occurred above those of *cantans* and the free swarms above or, more rarely, below them. Occasionally, the two were mixed together. We have also seen swarms consisting of a nucleus of mosquitos surrounded by Chironomids. The daytime resting places were the same as those of *Chaoborus* and, like the latter, the Chironomids could not be persuaded to form swarms during the daytime. The times of beginning and ending of swarming were much the same as in *cantans*. Swarms occurring later in the year were very irregular in their appearance; in some years they were observed during July and August, in others not at all. Sometimes they appeared every night for a week or so, at other times only on a single night. We only observed them for a prolonged period in 1939 and they differed somewhat from the earlier swarms. Very few individuals were seen during the daytime having regard to the large numbers participating in the morning and evening swarms and little was to be seen during the period of ascent. The swarms formed very suddenly and then remained high above the ground. They were all of the free type, often with a nucleus and sometimes with dependent columnar swarms. The start of swarming was very punctual. Swarms occurred every evening from the 5th to 24th August, 1939, and were first observed a quarter of an hour after sunset. They rose above the tree-tops when it was dark and stayed there for some time while the sound which they emitted gradually diminished. In this year we heard swarms on 27th, 28th and 30th August and 4th, 5th, 14th and 21st September. On all these occasions, the weather was calm. The observations were concluded at the end of September. It is possible that swarms may have been formed on nights other than those mentioned but that they were not heard on account of the noise made by the leaves. The scarcity of individuals during the daytime and the sudden increase in numbers in the evening coupled with the rapid disappearance of the morning swarms suggested that a fresh invasion of newly hatched individuals might be occurring each evening and that these might be returning to the lake to oviposit and die the following day. This is, however, pure speculation. An alternative explanation would be that the refusal to leave the shelter of the vegetation gave the impression of a smaller population than was actually the case.

Swarming of Culex pipiens.

A free swarm of this species was observed outside the laboratory at 17.12 hrs. on 20.x.1947. Small top-swarms of not more than four individuals each were observed in the usual places; the last of these disappeared at 17.41 hrs. On this occasion, the sky was unclouded and the wind S.W.4. The temperature at 17.30 hrs. was 3°C. at ground level, 6.5° at 2 m., 8° at 8 m. and 9° at 15 m. The following evening a few individuals were seen but there were no swarms. Temperatures ranged from -1.5° at ground level to 6° at 15 m. No mosquitos were observed on subsequent evenings.

Swarming of Tipulids.

A small swarm of Tipulids was observed on 26.v.1948 just below a free swarm of Culicids above the projector. They could easily be distinguished by the fact that they flew up and down vertically instead of round and round in a horizontal plane.

Relation of swarming to sunset and sunrise.

The close relation between the swarming of *Aedes cantans* and the times of sunset and sunrise has already been indicated. That the same holds good for *Chaoborus*, the Chironomids and *Culex pipiens* may be seen from fig. 9. Swarming in *Chaoborus*

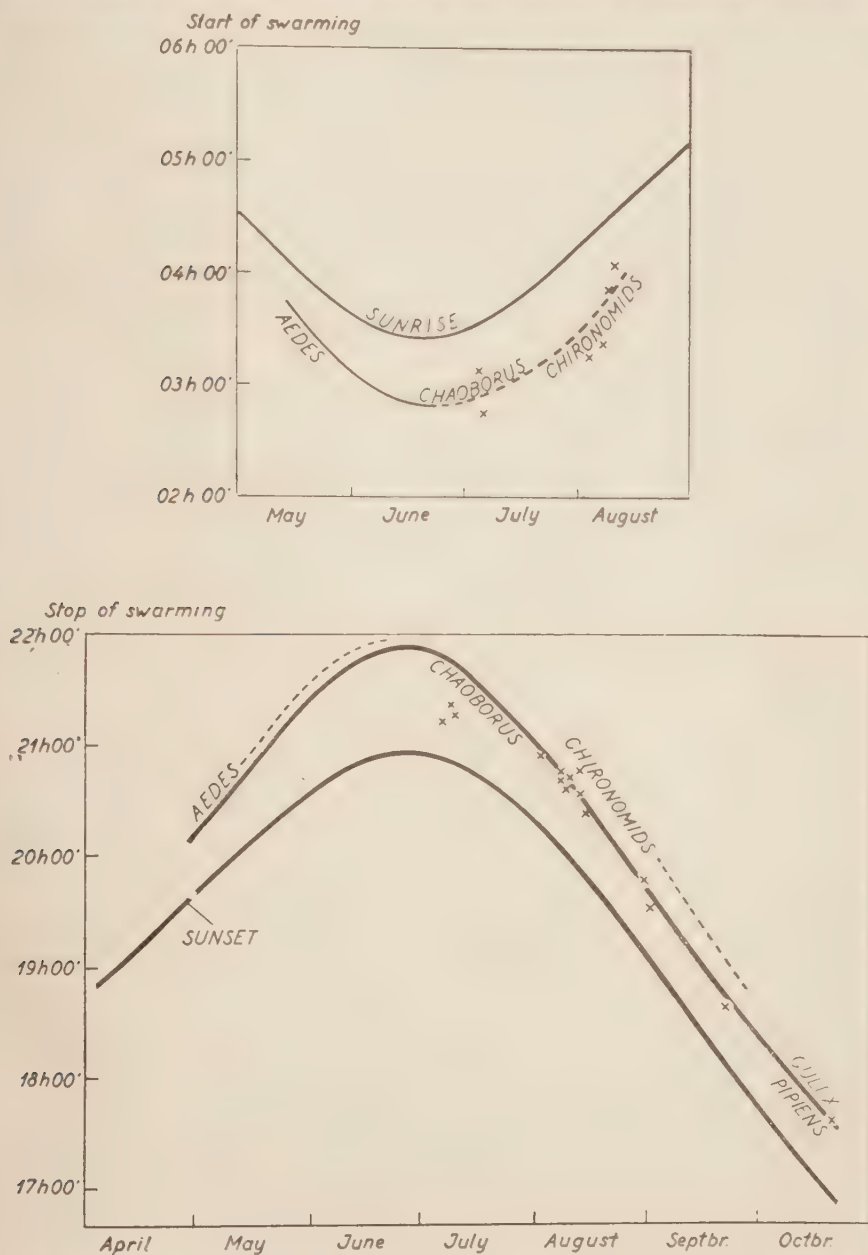


Fig. 9.—End of evening swarming and beginning of morning swarming in relation to sunrise and sunset.

appears to stop earlier than in the others and this point requires further elucidation. We were very strongly impressed by the regularity of swarming in the forms that we studied. It was evident that this was related to some factor or factors varying concurrently with the solar cycle and an attempt to explain this relationship led us to the detailed study of the ecology of swarming which is discussed below.

The Ecology of Swarming.

In order to reach a full understanding of the phenomena under discussion, it is clear that both the experimental approach and the method of observation in the field have to be employed. It is the purpose here to study the relationship between the times of morning and evening swarming and such physical factors in the environment as light intensity, temperature, humidity and wind velocity and to try and explain, as far as possible in terms of these factors, our observations in the field. The method was first to find the normal daily variation in each factor and then to examine closely those instances in which the variation departed from the normal. It appeared to us unlikely that the behaviour of the mosquitos depended on an internal rhythm but the possibility was not ignored and will be discussed below.

Light intensity.

As indicated above, the activity of the top-swarms ceases later in the evening as the year progresses (figs. 6 and 9, Table III). At the same time, the period of evening twilight (Sun $< 18^\circ$ below the horizon) grows longer and longer having its minimum duration at the equinox and its maximum at the summer solstice. The same is true of morning twilight and at Lat. 56° (the latitude at which the observations were made) morning and evening twilight overlap one another from 5th May to 8th August. Consequently, we could not use the variations in astronomical twilight and we therefore correlated the observations with the variations in civil twilight (Sun $< 6^\circ 24'$

TABLE V.
Light intensities in relation to swarming.
1946.

Date	20.00 hrs.		Start of top-swarms	21.30 hrs.		Dispersal of top-swarms	Light intensity at time of dispersal	
	Lux ₁₉₄₆	Lux ₁₉₄₇		Lux ₁₉₄₆	Lux ₁₉₄₇		Lux ₁₉₄₆	Lux ₁₉₄₇
28/5	290	74	19.58	19	8	21.25	25	9
5/6			20.29	19	8	21.25	23	8
6/6			20.22	50	18	21.46	20	7
7/6	360	88	20.18	54	19	21.39	23	8
8/6	420	99	20.15	20	9	21.26	24	8
9/6	250	55	20.17	37	14	21.32	27	9
10/6	330	82	20.27	47	17	21.47	21	7
11/6	370	90	20.26	68	23	21.46	24	8
12/6	340	84	20.12	54	19	21.45	22	8
13/6	320	80	20.05	47	17	21.43	21	7

below the horizon). It will be seen from the figures for the duration of evening twilight that there is good agreement with the observation that the time between sunset and the disappearance of the top-swarms increases from about 40 mins. in the middle of May to about 50 mins. in the middle of June. The actual times on 19th May, 16th June and 23rd June were 58, 70 and 70 mins., respectively. It appears very likely, therefore, that the top-swarms cease at a certain light intensity. As stated above, we met with considerable difficulty in measuring light intensity and, since different instruments were employed in 1946 and 1947, the standardisation of which, especially in 1946,

1947

Date	19.00 hrs.	Start of		21.00 hrs.	Dispersal of Top-swarms	Light intensity at time of dispersal
	Lux	Ascent	Top-swarms	Lux		
13/5	159	—	—	$\frac{1}{2}$	20.37	10
14/5	142	—	—	$\frac{1}{2}$	20.46	8
16/5	144	—	—	2	20.57	3
17/5	145	—	—	$3\frac{1}{2}$	21.01	3
18/5	145	19.36	20.22	—	20.32	—
19/5	146	19.05	—	$4\frac{1}{2}$	20.57	5
20/5	148	18.32	20.23	6	20.57	4
21/5	144	—	—	$6\frac{1}{2}$	20.58	5
22/5	140	18.00	19.55	6	21.15	2
23/5	144	19.12	19.43	—	21.25	—
24/5	—	19.00	19.15	$3\frac{1}{2}$	—	—
25/5	142	—	18.53	$13\frac{1}{2}$	21.23	3
26/5	154	—	18.35	27	—	—
27/5	156	19.05	19.56	$22\frac{1}{2}$	21.36	4
28/5	137	18.50	19.50	$19\frac{1}{2}$	—	—
29/5	133	—	19.43	$39\frac{1}{2}$	21.30	10
3/6	135	19.10	20.18	24	21.20	10
6/6	164	19.05	19.10	$37\frac{1}{2}$	21.31	10
9/6	150	19.49	19.59	30	21.40	5
11/6	136	—	—	$42\frac{1}{2}$	21.52	6
12/6	133	19.30	20.35	52	21.37	12
13/6	142	19.25	20.32	—	21.43	—
14/6	—	—	20.38	33	21.38	6
15/6	143	—	20.39	40	21.40	8
16/6	142	—	—	52	21.45	9

was somewhat uncertain, the two sets of figures are given separately (Table V). In 1946 there was no correlation between the beginning of swarming and the light intensity but there was a clear correlation between this factor and the time of cessation of the top-swarms. The stronger the light the later the mosquitos stopped swarming (fig. 10 *a, b*). There appeared to be no correlation between the light intensity and the

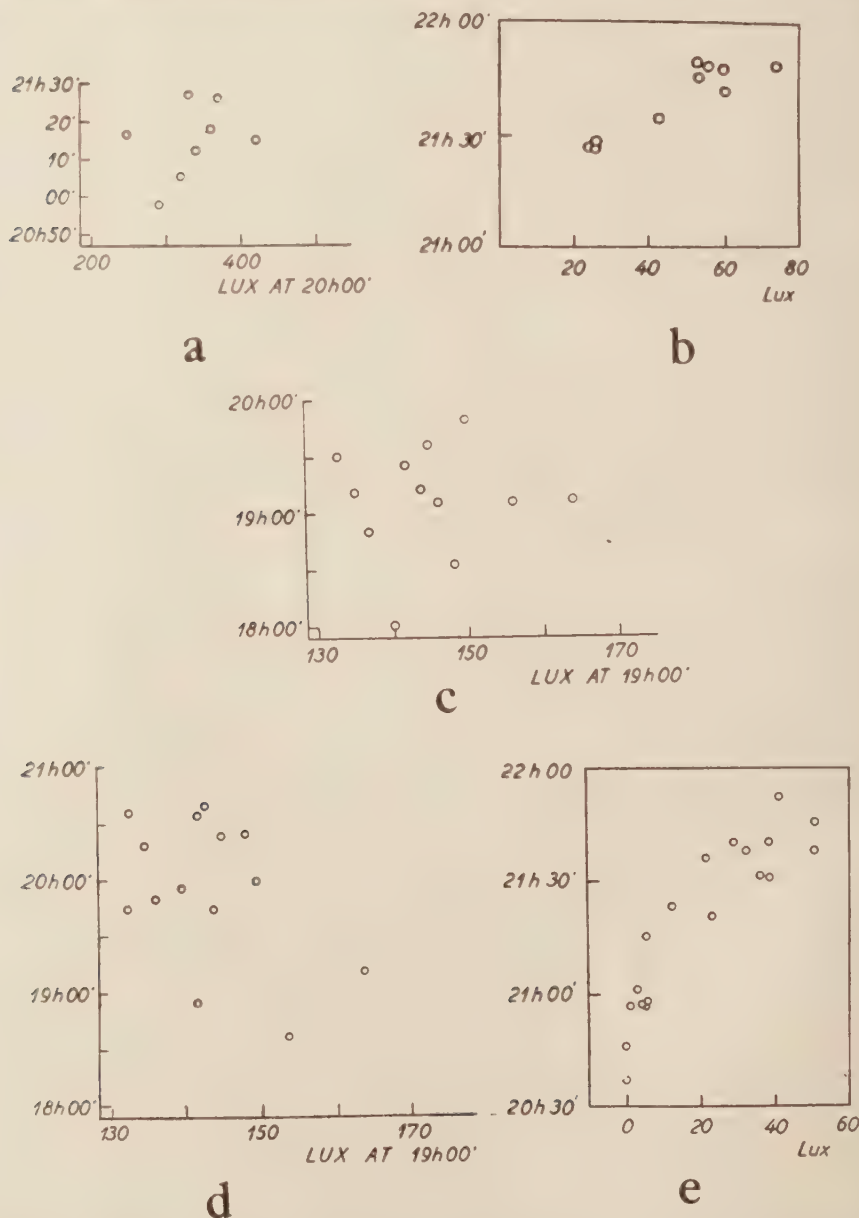


Fig. 10.—Relation of evening swarming to light intensity. (*a*) Start of swarming, 1946. (*b*) End of swarming, 1946. (*c*) Beginning of ascent, 1947. (*d*) Beginning of swarming, 1947. (*e*) End of swarming, 1947.

beginning of the ascent in 1947 (fig. 10 *c*) and, although there was some indication of a reverse correlation with the beginning of swarming (fig. 10 *d*), we considered this insufficient and preferred to seek another explanation (see below). There was again a strong correlation between the light intensity and the time of cessation of the top-swarms (fig. 10 *e*). In 1947, light intensities after 21.32 hrs. were calculated by extrapolation but it is not thought that the error involved can be very considerable. In order to compare the 1946 and 1947 figures, the mean light intensities for 20.00 hrs. and 21.00 hrs. were used (the former obtained by interpolation for 1947). These gave three points on which to base a curve, including the zero point (both instruments registered zero in darkness). The mean light intensity at which the top-swarms ceased in 1946 was 23 Lux. This would correspond to 8 Lux from the curve as measured by the 1947 instrument which is in reasonable agreement with the mean value of 6.5 Lux actually found in 1947. It appears, therefore, that the swarming of mosquitos actually stopped at about 7 Lux₁₉₄₇. The small number of observations which were made on light intensities at the time of morning swarming agreed fairly well with those made in the evening. Swarming began in slightly stronger light than that which marked the ending of the evening swarming but this may have been because the mosquitos rose in swarms from the shade of the foliage in which they were resting.

Temperature.

The variation of temperature with height above the ground on a typical night in early summer is shown in fig. 11. On the occasion in question, the day had been fine with a light breeze from the S.E. which subsided about 19.00 hrs. There was no cloud. At 18.00 hrs. the sun was still shining on the heather at the foot of the mast and the temperature, as always during the daytime, was highest on the ground. At 19.00 hrs. the sun had ceased to shine on the heather but was still shining on the mast. The highest temperature was now recorded at 5 m. above the ground. At 20.00 hrs.

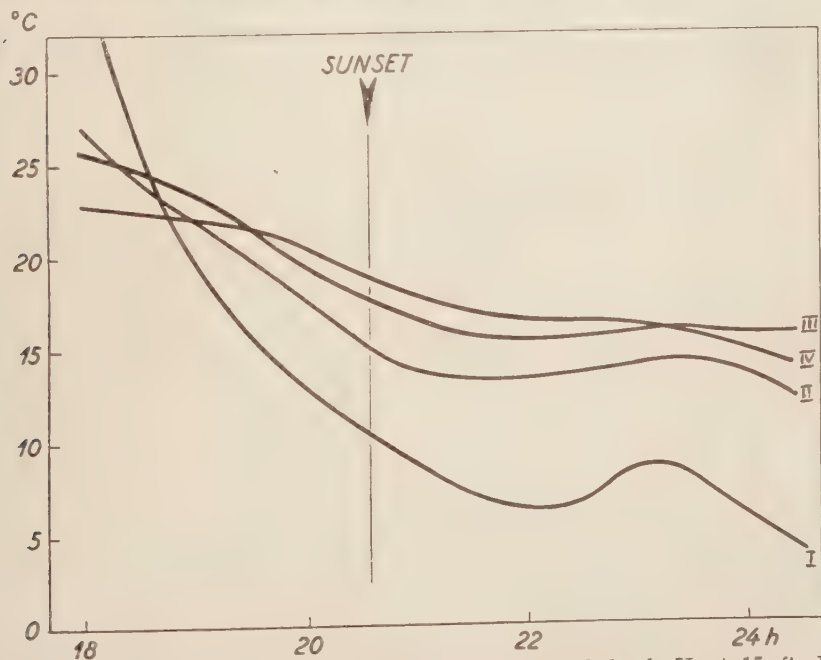


Fig. 11.—Temperatures on 29th May, 1946. I at ground level. II at 15 ft. III at 30 ft. IV at 45 ft.

the typical night-time distribution was recorded with the temperature lowest at ground level and steadily increasing with height. Astronomical sunset occurred at 20.34 hrs. and at 21.00 hrs. a drop in temperature of about 4–5°C. at all heights was recorded. Towards 22.00 hrs., however, the fall in temperature became slower especially near the ground where, shortly afterwards, it began to rise again. This is a curious phenomenon which is termed the "hothouse effect". It has received little attention in the literature (see, however, Geiger 1942, pp. 25, 67). It is discussed in more detail below. A little before midnight the "hothouse effect" ceased and the temperature fell until sunrise. Such a picture is characteristic of calm, clear weather and it was for this reason that we chose the occasion in question for purposes of illustration. In cloudy or windy weather the picture becomes distorted and more or less atypical. Various explanations for the "hothouse effect" occurred to us but all except one were discarded. It is well known that the evening fall in temperature may be brought to a halt when the dew point is reached in consequence of the release of heat resulting from condensation; this cannot, however, possibly produce a rise in temperature. It is possible that loss of water vapour in the lower layers of the atmosphere might cause a contraction in volume and a consequent influx of warm air from the upper layers but any increase in temperature from this cause would be much smaller than that which was actually observed. Since the increase comes earlier and is greater close to the ground than higher, it seems to us that the cause must be sought in the ground. In our opinion, the most probable explanation appears to lie in the well-known fact that long-wave radiation from the earth's surface is arrested to a large extent by humid air. Some may even be returned as the "Gegenstrahlung" of Geiger (1942, pp. 17–28). A few inches below the surface the ground is still warm after the daytime insolation and the reduction of radiation from the surface will cause the temperature here to rise.

A few laboratory observations have been made on the temperature relationships of *Culicids* but all that appears to have been established is that they tend to avoid temperatures above 24°C. or below about 4–6°C. at which temperature they are to some extent immobilised. Table VI shows the temperatures at the time of the evening ascent taken actually in the heather or as near as possible to the site of the ascent.

TABLE VI.
Relation of temperature to time of evening ascent.

Date 1947	Time of ascent	Temperature at time of ascent		Date 1947	Time of ascent	Temperature at time of ascent	
		Heather	Screen			Heather	Screen
18/5	19.36	10°C.	11°C.	31/5	19.00	21°C.	28°C.
19/5	19.05	11°C.	15°C.	3/6	19.10	15°C.	18°C.
20/5	18.32	17°C.	20°C.	6/6	19.05	17°C.	16°C.
22/5	18.00	19°C.	21°C.	7/6	20.05	12°C.	14°C.
23/5	19.12	13°C.	22°C.	8/6	20.06	11°C.	11°C.
24/5	19.00	15°C.	18°C.	9/6	19.49	7°C.	15°C.
27/5	19.05	17°C.	20°C.	12/6	19.31	12°C.	17°C.
28/5	18.50	14°C.	18°C.	13/6	19.25	12°C.	17°C.
30/5	19.05	19°C.	26°C.				

The temperature figures measured in the weather screen are given in the last column. It will be seen that considerable errors would have been introduced had we relied on the latter alone. Figure 12 indicates that there is a clear correlation between temperature and the time of ascent. The latter takes place soon after 18.00 hrs. in warm weather and may be delayed up to two hours in the cold. Passing from the ascent to the time of commencement of the top-swarms, there is again a good correlation with temperature (Table VII, fig. 13). Here, however, the dispersion is somewhat

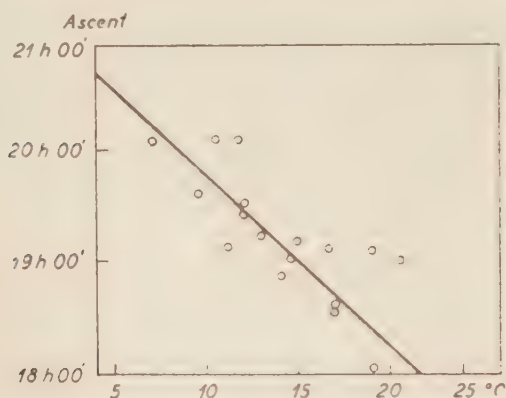


Fig. 12.—Correlation between time of evening ascent and temperature as measured in the heather.

TABLE VII.

Relation of temperature to time of commencement of top-swarms in the evening.

Date	Time	T. (°C.)	Date	Time	T. (°C.)	Date	Time	T. (°C.)
1940			1946			1947		
21/5	19.55	17	3/6	20.30	13	27/5	19.56	18
22/5	19.52	18	4/6	20.24	13	28/5	19.50	15
23/5	19.52	18	5/6	20.29	15	29/5	19.43	21
25/5	19.35	17	6/6	20.22	13	3/6	20.18	13
26/5	19.40	18	8/6	20.15	16	5/6	20.28	17
27/5	19.55	15	10/6	20.27	15	6/6	19.10	16
28/5	19.28	15	11/6	20.27	12	9/6	19.59	12
29/5	19.25	15	12/6	20.12	15	12/6	20.35	12
30/5	19.50	15	13/6	20.30	13	13/6	20.32	13
31/5	19.40	15	20/6	20.14	13	14/6	20.38	11
1/6	19.40	15	21/6	20.12	14	15/6	20.39	11
2/6	19.40	15	1947			17/6	20.41	13
3/6	20.03	18	18/5	20.22	9	18/6	20.46	16
6/6	20.37	18	20/5	20.23	18	1948		
9/6	20.48	15	22/5	19.55	12	9/5	19.00	18
1946			23/5	19.43	16	10/5	18.53	20
26/5	19.50	18	24/5	19.15	15	11/5	18.27	21
27/5	19.50	17	25/5	19.33	16	16/5	18.45	18
28/5	19.58	18	26/5	19.35	19	20/5	19.11	14

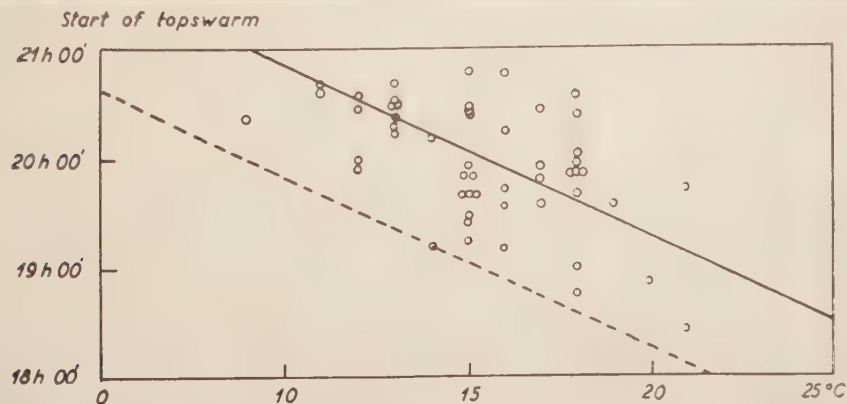


Fig. 13.—Correlation between temperature (at 25 ft. above the ground) and time of commencement of top-swarms. The dotted line shows the time of the ascent and has been transferred for comparison from fig. 12.

larger and the impression was gained that the top-swarms normally formed about one hour after the ascent and thus showed a similar relation to temperature but that the precise length of the interval was perhaps governed by other factors. Table VIII and fig. 14 show temperatures at the time of dispersal of the top-swarms. Here the

TABLE VIII.
Relation of temperature to time of dispersal of top-swarms in the evening.

Date.	Time.	T. (°C.)	Date.	Time.	T. (°C.)	Date.	Time.	T. (°C.)
1940			1946			1947		
20/5	20.39	13	28/5	21.25	15	22/5	21.15	8
21/5	20.55	15	29/5	21.25	16	23/5	21.25	13
22/5	21.08	15	30/5	21.25	17	25/5	21.23	9
23/5	21.17	16	2/6	21.35	13	27/5	21.36	15
24/5	20.57	15	3/6	21.34	12	29/5	21.30	14
25/5	21.18	15	4/6	21.40	12	3/6	21.20	10
26/5	21.30	15	5/6	21.25	14	6/6	21.31	9
27/5	20.35	13	6/6	21.46	12	9/6	21.40	12
28/5	21.13	14	9/6	21.32	13	11/6	21.52	11
29/5	21.18	14	10/6	21.47	15	12/6	21.37	9
30/5	21.28	13	11/6	21.46	11	13/6	21.43	8
31/5	21.23	14	1947			14/6	21.38	10
1/6	21.33	14	12/5	20.36	13	15/6	21.40	13
2/6	21.30	14	13/5	20.37	14	16/6	21.45	13
3/6	21.43	16	14/5	20.46	15	17/6	21.52	10
4/6	21.29	12	15/5	20.47	14	18/6	21.52	13
5/6	20.59	16	16/5	20.57	14	1948		
6/6	21.55	16	17/5	20.01	15	9/5	20.52	16
7/6	21.52	14	18/5	20.32	9	10/5	20.52	17
8/6	21.56	15	19/5	20.57	6	11/5	20.50	15
9/6	21.59	15	20/5	20.57	13	16/5	20.46	16
10/6	21.36	14	21/5	20.58	10	20/5	20.15	13

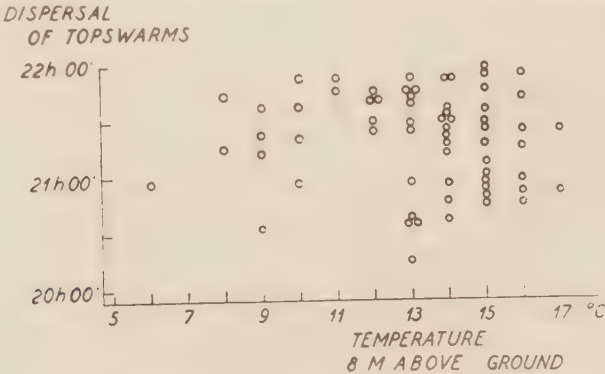


Fig. 14.—Temperature at time of dispersal of top-swarms.

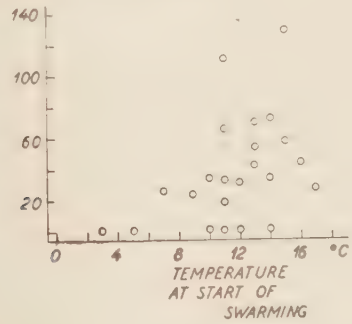
governing factor appeared, as indicated above, to be light and accordingly there was no apparent correlation with temperature. There are much fewer observations on the morning swarms, but the available data are given in Table IX. There is no obvious relation to temperature but we feel that this may be masked by the dependence of the commencement of swarming on changes in light intensity. The best way of discounting this influence seemed to us to be to consider the duration of the swarms rather than the times of their commencement and dispersal. Figure 15 shows the duration of the morning swarms plotted against the temperatures at the beginning and ending of swarming, and the total duration of swarming plotted against the

TABLE IX.

Temperatures associated with commencement and dispersal of top-swarms in the morning.

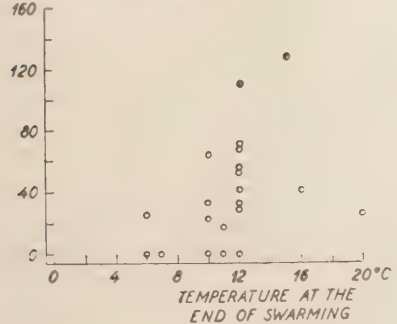
Date	Top-swarms began.		Top-swarms ended.		Date	Top-swarms began.		Top-swarms ended.	
	Time.	T. (°C.)	Time.	T. (°C.)		Time.	T. (°C.)	Time.	T. (°C.)
1946					1947				
27/5	02.47	13	03.56	13	3/6	03.15	16	03.57	16
1947					8/6	03.15	12	03.45	12
18/5	03.30	(11)	04.35	10	9/6	03.05	9	03.27	10
19/5	03.35	7	04.00	6	10/6	02.50	11	04.20	12
24/5	—	—	05.04	(10)	15/6	02.46	11	03.18	12
26/5	—	—	04.25	9	16/6	02.58	13	03.39	12
28/5	03.20	13	04.13	12	17/6	03.10	11	03.28	11
29/5	—	—	04.13	12	18/6	03.10	17	03.36	20
30/5	02.57	10	—	—	19/6	03.12	14	03.45	—
31/5	03.00	15	05.08	17	1948				
1/6	—	—	04.08	11	7/5	03.51	10	04.24	10
2/6	02.58	15	03.54	12	10/5	03.52	14	04.43	12

DURATION OF TOPSWARM



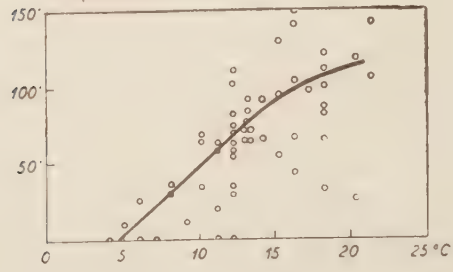
a

DURATIONS OF TOPSWARMS



b

Minutes duration of topswarm



c

Fig. 15.—Correlation between temperature and duration of top-swarms. (a) Duration of morning swarms plotted against temperature at the start of swarming. (b) Duration of morning swarms plotted against temperature at the end of swarming. (c) Total duration of swarming (evening and morning taken together) plotted against temperature at the start of swarming in the evening and at the end of swarming in the morning.

temperature at the commencement in the evening and the end of swarming in the morning. It appears evident that swarming continues for a shorter time when it is cold and a longer time when it is warm. If the temperature is below about 10°C., the duration will become zero, *i.e.*, there will be no swarming at all; this corresponds well to observations in the field and in the laboratory. The importance of the temperature is in determining the time of commencement in the evening and the time of dispersal in the morning.

Humidity.

The habit of swarming at dusk and dawn, and of avoiding sunshine, displayed by the species which we studied led us to anticipate a close relationship between humidity and the times of swarming, as indicated above, our humidity measurements were not very precise but the Assmann psychrometer should have been sufficiently accurate to show a correlation if such existed. It appeared, however, that it did not (Table X).

TABLE X.

Relation of humidity to time of ascent and times of commencement and dispersal of top-swarms in the evening (1947).

Date	Time of ascent.	Top-swarms began.	Rel. Hum.	Sat. Def.	Top-swarms ended.	Rel. Hum.	Sat. Def.
18/5	19.36	20.22	—	—	20.32	67.0	2.76
19/5	19.05	—	—	—	20.57	95.6	0.29
20/5	18.32	20.23	68.3	5.26	20.57	—	—
22/5	18.00	19.55	42.1	7.13	21.15	70.3	2.45
23/5	19.12	19.43	44.5	7.44	21.25	—	—
24/5	19.00	19.15	64.2	4.81	—	—	—
25/5	—	18.53	63.2	5.00	21.23	92.6	0.66
26/5	—	18.35	65.5	5.09	—	—	—
27/5	19.05	19.56	89.0	1.73	21.36	92.7	0.90
28/5	18.50	19.50	72.7	3.79	—	—	—
29/5	—	19.43	64.7	6.36	21.30	87.2	1.71
1/6	—	—	—	—	21.52	68.9	4.46
3/6	19.10	20.18	72.4	3.77	21.20	93.8	0.54
6/6	19.05	19.10	68.0	3.91	21.31	86.7	1.40
9/6	19.49	19.59	65.7	4.04	21.40	94.8	0.41
11/6	—	—	—	—	21.52	75.2	2.04
12/6	19.30	20.35	69.4	3.85	21.37	96.1	0.33
13/6	19.25	20.32	56.0	5.85	21.43	90.9	0.72
14/6	—	20.38	79.8	2.09	21.38	97.4	1.23
15/6	—	20.39	96.6	0.36	21.40	97.6	0.24
16/6	—	—	—	—	21.45	66.3	3.93

Only in the case of the time of ascent, however, was there any apparent correlation and this disappeared when allowance was made for temperature.

Wind.

We have already shown above (p. 240) that strong winds modify the character of the swarms and the flight of the individual mosquitos considerably. In 1939, we had three evenings with wind strengths of more than 3 Beaufort and on each occasion swarming ceased extremely late. Accordingly we formed the opinion that strong winds have a delaying effect on the dispersal of the top-swarms. Subsequent observations failed to confirm this, however, and we were forced to conclude that the apparent relationship was purely coincidental.

Rain.

Light rain during swarming appeared to have no effect. Sudden increases in the intensity of the downpour may depress the swarm vertically for two or three feet

but the effect is momentary. A combination of rain with low temperature and high winds may shorten the duration of swarming or even inhibit it altogether.

Ozone.

We made no attempt to measure the concentration of this substance in the atmosphere, nor had we any particular reason to believe that it influenced the behaviour of the mosquitos. But, on a number of occasions, as for example between 03.03 hrs. and 03.13 hrs. on 1.vi.1947, we noticed a strong smell of ozone apparently emanating from the foliage where the mosquitos were harbouring. This phenomenon was observed on calm nights and mostly between dawn and sunrise. We have no explanation for it but it appeared to us that it must be associated with some difference between the nights on which it occurred and those on which it did not and that this difference might not be without effect on the behaviour of the mosquitos.

Discussion.

It was said many years ago by C. O. Whitman that biology is made up of equal parts of observation, experiment and reflection. Our experiments, of which a short account is given below, have been too few to permit of any lengthy reflections on the problems with which we were concerned. It seemed to us, however, desirable to discuss briefly the results of our observations and to suggest certain lines of enquiry that may prove profitable when an experimental investigation is undertaken. The question "Why do the mosquitos swarm?" involves two different problems concerning respectively the biological significance of swarming and the factor or factors by which it is initiated. With regard to the first of these problems, we do not claim to have arrived at any final solution but we hope to have corrected the erroneous impression that the swarms are mating swarms.

It will be seen from the figures given that at night the temperature near the ground may be very low and it might be that swarming represents an escape from this low temperature. This would, however, in no way explain the formation of swarms by the males on their way to the tree-tops while the females seem to suffer no harm from remaining near the ground later in the season. The most probable explanation seems to us to be that swarming serves to prevent inbreeding by mixing males from different breeding places. The females appear on the whole to stay in much the same place all their lives and to lay their eggs in the breeding places from which they themselves emerged and the swarming habit provides them with an opportunity to mate with males from other breeding places. The tenability of this hypothesis could be tested by means of marked mosquitos. With regard to the second problem it is clear that the activating factor cannot be temperature. It has been shown above (p. 250) that low temperatures may delay the start of swarming or even totally inhibit it but the fact that the same temperature may persist during the whole afternoon and evening or the daytime temperature may on occasion fall to the evening level without producing any tendency to swarm appears to preclude it as the causative agent. On the other hand, we have on a few occasions noticed the daytime swarms well outside the normal swarming times and apparently resulting from a fall in light intensity associated with approaching rain. This is particularly true of small Chironomids but we observed at least one such swarm of *Aedes cantans* (between 13.55 and 14.10 hrs. on 24.v.47). Again on 8.v.48 when rain started at about 18.25 hrs., top-swarms appeared above several of the lower trees while the ascent started over the whole area. A few moments later the sun started to shine, although the rain persisted, and at once the swarms were much reduced in size. This lasted until 18.45 hrs. when the rain ceased. Between 18.50 and 19.25, the ascent took place in the usual way and the swarms developed normally until the disappearance of the top-swarms at 20.37. These observations suggested to us that light was possibly the causative agent. We have shown above (p. 249) that swarming activity ceases at

TABLE XI.
Results of a typical experiment on response to change in light intensity.

Time	No. on wing	Average	T. & R.H.	Time	No. on wing	Average	T. & R. H.
10.07.00	3	3.5	63°F. 95%	10.13.00	16	22.0	63°F. 95%
15	4			15	24		
30	4			30	20		
45	3			45	28		
10.08.00	6	6.5		10.14.00	23	21.3	
15	6			15	19		
30	7			30	21		
45	7			45	22		
10.09.00	7	5.5		10.15.00	18	17.0	
15	5			15	15		
30	6			30	16		
45	4			45	19		
10.10.00	3	3.5		10.16.00	20	16.5	
15	2			15	13		
30	3			30	16		
45	6			45	17		
10.11.00	3	4.5		10.17.00	11	12.5	
15	6			15	11		
30	5			30	12		
45	4			45	16		
Total ...	94	4.7			357	17.9	

Mosquitos caught at 20.00 hours on 16.v.48 were kept in darkness until 08.15 hours on 17.v.48. They were then kept at a light intensity of 6 Lux which was suddenly increased to 100 Lux at 10.13 hours.

light intensities below 7 Lux but we could find no correlation between light intensity and the commencement of swarming. We were, therefore, led to suppose that the factor involved was a change in light intensity rather than a threshold effect and some support was given to this hypothesis by certain experiments. We had previously carried out in our laboratory experiments on the behaviour of larger insects such as grasshoppers (Nielsen, 1937), Hymenoptera (Nielsen, 1945) and Noctuids and we devoted a considerable amount of time to modifying the same techniques for use with mosquitos. We obtained some success with a method involving the recording of a light spot on photosensitive paper on a kymograph but the apparatus was so sensitive that we could use only one mosquito at a time. It appeared that activity was increased by a change of light intensity in either direction. In 1947 we employed another technique. A considerable number of mosquitos, usually about 200, were liberated in the constant temperature room which is about 160 x 180 cm. in area and 200 cm. high. On the floor there were tubs planted with grass and in the centre a small birch tree about 1 m. high. Most of the room could be seen through a window and outside there were mechanisms for the regulation of light, temperature, humidity and air movements while inside were thermometers, hygrometers and a Luxmeter of the type described above. Every 15 seconds we counted the number of mosquitos on the wing and, in general, we took the mean of 20 such counts as representing the degree of activity under the conditions in question. The method proved practicable and gave some interesting results but we were unable to find time for many experiments while we were engaged in the field work. We continued the experiments in 1948 but analysis of the data proved very difficult and when we left it the work was far from complete. Some tentative conclusions follow.

1. When the light was kept constant no periodic activity was observed which would indicate a 24-hour cycle.

2. Except as indicated below, an increase in activity accompanied every change in light intensity. The greater the change in light intensity, the greater was the change in activity.

3. Sometimes there was no change in activity accompanying a change from higher to lower light intensity. We could find nothing to account for these exceptions.

4. There appeared to be an adaptation to changing light intensity since the increased activity gradually diminished following the change in light intensity until the original level was reached after 15–30 minutes (see Table XI).

5. A fresh change in light intensity, a few minutes after the normal level of activity was reached, produced a fresh change in activity. It appeared to be immaterial whether or not the second change in light intensity was in the same direction as the first. The change in activity produced by changes in light intensity resembles in some respects the klinokinesis in the reflex system demonstrated by Fraenkel and Gunn (1940). It does not suffice wholly to explain the swarming activity and we only occasionally observed one or two individuals flying above the birch tree in a manner suggestive of swarming.

All that can be said concerning these experiments is that they appear to indicate that the general activity of mosquitos is influenced by changes in light intensity and that there is no evidence of an internal rhythm. A final point requires emphasis, namely that our statements in this paper regarding behaviour in the field refer to swarms and not to individual mosquitos. The commencement of swarming has been understood by us to be the appearance of the first mosquito and the disappearance of the top-swarms we have taken to mean the disappearance of the last mosquito in the swarm which may take place more than an hour after the first mosquitos have already retired. It appears to be necessary to assume a considerable variation in the sensitivity of individual mosquitos to explain this. We have no experimental evidence bearing on this point but our field observations gave some support to such an assumption.

Summary.

The principal object of this work was to elucidate the swarming behaviour of *Aedes cantans* and other mosquitos, *Chaoborus crystallinus* and certain Chironomids. In addition, ancillary studies were made of the general ecology of these species. The swarms were found to consist entirely of males and to bear no direct relationship to mating or to the search for food. There was no noticeable difference between the swarming habits of the different species of mosquitos and even the differences between the Culicids and Chironomids were very slight. Swarming was observed to take place at about sunset in the evening and sunrise in the morning. The evening swarms appeared to be formed in response to decreasing light intensity and to disperse at a light intensity of about 7 Lux. Low temperatures may delay the start of swarming. The morning swarms started at about the same threshold of light intensity and at this time also their duration was reduced by low temperatures. Below about 50 F. they were not formed at all. Atmospheric humidity appeared to be of minor importance.

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References.

- FRAENKEL, G. S. & GUNN, D. L. (1940). *The Orientation of Animals: Kineses, Taxes and Compass Reactions.* Oxford.
- GEIGER, R. (1942). *Das Klima der bodennahen Luftschicht.* 2nd Edn. Brunswick.
- HOWARD, L. O., DYAR, H. G. & KNAB, F. (1912-17). *The Mosquitoes of North and Central America and the West Indies.* Washington, Carnegie Inst.
- LARSEN, E. B. (1948). Observations on the Activity of some Culicids.—Ent. Medd., **25**, pp. 263-277.
- MARSHALL, J. F. (1938). *The British Mosquitoes.*—London, British Museum (Nat. Hist.).
- MEINERT, F. (1886). De eucephale Myggelarver.—K. danske vidensk. Selsk. Skr., **3**, pp. 373-493.
- NIELSEN, E. T. (1937). Zur Oekologie der Laubheuschrecken.—Ent. Medd., **20**, pp. 121-164.
- NIELSEN, E. T. (1945). *Moeurs des Bembex.* Monographie biologique avec quelques considérations sur la variabilité des habitudes.—Spolia zool. Mus. haun., **7**, pp. 1-174.
- WESENBERG-LUND, C. (1921). Contributions to the biology of the Danish Culicidae.—K. danske vidensk. Selsk. Skr., **7**, pp. 1-210.
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The study area from the air (about 650 ft.) looking East. 1—Cultivated area. 2—Western end of "Valdemarsvej". 3—Old vegetable garden. 4—Weather Screen. 5—Heather. 6—Laboratory. 7—Projector. 8—Road ("Store Ryvej"). 9—Grassy plain, partly cultivated. 10—Grassy plain with birches. 11—Peat ditch. 12—Birch wood. 13—Closed path. 14—Reed swamp. 15—Road ("Morten Larsensvej"). 16—Birch wood. 17—"Valdemarsvej". 18—Heather. 19—Old peat ditches. 20—Road ("Lille Ryvej"). 21—Peat ditch. 22—Meadow. 23—Entrance drive to Laboratory. 24—Observation mast. 25—Boundary ditch.—*Photo by Lufffoto Nowico, Copenhagen.*



Aedes cantans in daytime resting and feeding places: (a), ♀ and (b), ♂ in grass; (c), ♀ on catkin of *Salix pentandra*; (d), ♂ on whitethorn.

THE PERSISTENCE OF TOXICITY IN DDT-IMPREGNATED HESSIAN AND ITS USE ON TSETSE TRAPS.

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Extensive tests with traps of the "animal" pattern against the riverine tsetse, *Glossina palpalis* (R.-D.) and *G. tachinoides* Westw. have shown that their use can effectively reduce the population of flies in isolated patches of flybelt such as sacred groves. The incidence of flies at feeding-grounds in continuous flybelt can be considerably lowered, and kept low, by the strategic placing of relatively small numbers of traps. Where human beings are constantly present in such environments and in particular when day after day the same individuals visit the same place such as a water-hole in a grove, or a river bank for washing or watering cattle, conditions are most favourable for the transmission of trypanosomiasis. It is at such places that traps can play an important role in protection. The reduction in fly incidence is more important than it appears because the irritation caused to their hosts makes it likely that the flies have to pass from one host to another in their attempts to obtain a full meal before coming to rest on a passive trap; thus the potentially infective flies are being selectively drained off. Any increase in the efficiency of traps is, therefore, a desirable objective (Morris & Morris 1949).

Additional efficiency in trapping might be attained in two ways, by increased knowledge of the habits of the flies or by an intrinsic improvement in the traps. It was considered that the latter might be obtained by treating the traps with an insecticide having a residual action. DDT was an obvious choice, and by good fortune Professor P. A. Buxton brought samples when he visited Lawra in 1944. Professor Buxton carried out an initial experiment in which contacts of one minute with DDT-impregnated cotton resulted in a high rate of mortality within a few hours. Later, trials carried out by K. R. S. Morris with hessian soaked in a saturated solution of DDT in kerosene showed that brief contacts of 10-15 seconds were sufficient to cause mortality in tsetse. Even after five months' exposure to the weather, contact with this material was still able to cause mortality in a proportion of the flies. But, owing to the deaths of flies amongst the controls overlapping those dying with symptoms of poisoning, it was impossible to arrive at an accurate assessment of loss of efficiency.

If traps are to be of value for protection against trypanosomiasis, it must be possible to employ them over extensive areas and they must be simple to maintain and reliable. Moreover, if the traps are to incorporate the use of an insecticide, it is essential to know, accurately, the effect of the particular application in use and how long it can be depended upon under conditions of exposure to weather. These matters are extremely difficult to determine in the field, yet they are of vital importance. For this reason, a series of investigations was undertaken with the object of measuring exactly, in terms of fly mortality, the initial toxicity of DDT-impregnated hessian and the subsequent duration of toxicity under normal weather conditions. The variability of the weather conditions encountered gave unexpected results, and this led to the development of a technique of double controls as providing the only means of arriving at definite conclusions. The work was then related to practical usage by field tests with impregnated traps, the results of which were definitely encouraging.

Preliminary Tests.

Method.

In these experiments DDT manufactured by Messrs. Geigy was used. The DDT was dissolved in kerosene by being shaken vigorously in a bottle until no more would dissolve and the resulting solution filtered to free it from impurities. This was usually carried out at afternoon shade temperatures of about 85 F. with the result that, by the following morning, the filtrate showed needle-shaped crystals of pure DDT which were readily re-dissolved by further shaking. Hessian, which was otherwise satisfactory as a covering for animal traps, was considered to be a material likely to hold DDT well. It was also thought that soaking the cloth in the kerosene solution would allow a more thorough penetration of the DDT than was likely to be obtained from spraying.

The tests for the persistence of toxicity were begun in September, 1946. A square foot of new hessian was soaked for ten minutes in a DDT solution; during the process the cloth was moved about in the liquid to assist the even distribution of the insecticide. It was then hung up to dry over-night on the laboratory veranda; by next day it was dry but still smelt strongly of kerosene. The colour was slightly lighter than that of the untreated cloth but the DDT was not obvious. With the exception of a small piece which was cut off and hung in the shade of the veranda, the impregnated hessian was hung by wire loops from the branch of a small tree in such a position that it was fully exposed to the weather and in particular was freely blown about by the prevailing winds.

All flies used for tests were obtained on the day needed from flybelt on the Black Volta River 3 miles away. They were caught by skilled flyboys who could handle them without injury. Operations were started early in the morning, and batches of 25 or so flies as caught were brought to the laboratory with the minimum of delay and immediately released into a gauze-covered cage of $\frac{3}{4}$ cubic foot capacity, fitted with a sleeve at each end. The flies were transferred individually from the cage to 6 x $\frac{3}{4}$ ins. test-tubes lightly plugged with cotton-wool. The numbers of flies used in the tests were sometimes small, but the flyboys did their best in times of scarcity. It was not possible to restrict the flies chosen for the experiments to any one category such as males or females, old or young; all healthy and active specimens of *G. tachinoides* were used. There was a wide range in the state of hunger of the flies, some of them being very fully fed by the time they were isolated in the test-tubes. It was observed that while a recent meal probably extended the life of a control fly it appeared to have little effect on the time it was likely to survive after contact with DDT.

The impregnated cloth was spread flat on thick paper during tests to avoid contamination of the bench. This is important where accommodation is limited, and it is even more important to remember that whoever is responsible for sorting the flies into the test tubes must not be allowed to help with the actual testing, and most certainly must not have taken part in the preparation of impregnated material. Under the primitive conditions of a bush laboratory, it was found impossible to ensure adequate cleansing of insecticide from the boys' hands. The tsetse were brought into contact with the insecticide by inverting each test-tube on the cloth and tapping gently to knock the fly down on to the cloth where it was left for a definite number of seconds, 15 or 60 in the first series of tests. It was then removed by slipping a small card between the cloth and the mouth of the tube and the cotton wool replaced. The easiest way to time the contact with the insecticide was found to be by counting the seconds and, if a fly left the cloth, to discontinue until it was again in place. This method of exposing the flies individually for a short space of time was adopted in all the tests in preference to leaving them in more or less continuous contact until all were either paralysed or dead. The success of DDT-impregnated hessian in the field must depend on the effect on the tsetse of relatively brief contact so it was considered that the former method would give the more useful results.

TABLE I.

Summary of results of preliminary experiment to test the persistence of toxicity in DDT-impregnated hessian.

	Maxi- mum Tem- pera- ture °F.	Rela- tive Hu- mid- ity (8 a.m.) %	Exposure of Cloth to Weather		Interquartile Range and Median Values of Tsetse Survival		
			Days	Total Rain in inches	Tsetse in Contact with DDT for		Controls
					15 seconds	60 seconds	
<i>Hessian fully exposed to weather</i>					hrs.	hrs.	hrs.
1946							
1 Oct.	84	91	—	—	2.2-2.9-3.5	— — —	8.2-12+ —
2 "	81	87	1	None	2.5-4.2-6.2	3.6-3.8-4.8	11.5-12+ —
3 "	83	83	2	"	4.4-5.8-6.7	3.3-4.7-7.7	10.8-12+ —
7 "	86	91	6	1.43 on 3 days	4.9-5.5-6.1	3.7-4.4-6.8	12+ — —
14 "	87	91	13	2.71 " 7 "	6.0-7.5-9.6	3.3-5.4-6.0	12+ — —
15 "	81	87	14	2.71 " 7 "	4.9-5.7-10.6	3.9-6.6-8.8	12+ — —
21 "	89	83	20	3.09 " 8 "	4.2-5.4-7.6	3.8-4.6-6.6	9.0-12+ —
22 "	88	83	21	3.09 " 8 "	5.1-7.3-12+	3.0-4.6-5.6	10.3-12+ —
28 "	90	79	27	3.91 " 10 "	3.8-6.0-6.8	4.4-5.4-6.3	6.3-12+ —
9 Nov.	92	79	39	4.26 " 11 "	2.9-4.4-6.8	3.8-4.5-6.3	7.6-10.2-12+
23 "	92	75	54	4.26 " 11 "	2.4-2.9-4.9	2.0-2.7-3.5	4.1-5.4-9.6
7 Dec.	92	75	68	4.26 " 11 "	2.3-3.0-4.7	1.7-2.3-3.4	4.8-8.2-9.4
21 "	95	45	82	4.26 " 11 "	2.3-2.6-3.3	2.3-2.5-3.0	3.2-5.1-7.2
1947							
18 Jan.	91	55	110	4.26 " 11 "	2.7-3.1-3.9	2.7-3.2-3.8	5.2-6.7-12+
21 Feb.	95	75	144	4.26 " 11 "	3.0-4.1-6.4	2.8-4.1-5.8	7.0-8.6-12+
28 Apr.	99	60	210	4.26 " 11 "	6.4-9.0-12+	6.4-8.0-8.9	8.0-12+ —
12 Sept.	81	76	347	34.35 " 67 "	12+ — —	8.1-11.8-12+	12+ — —
<i>Hessian protected in veranda*</i>							
1947							
28 Apr.	99	60	210	—	3.7-4.7-6.5	4.5-5.1-6.8	8.0-12+ —
12 Sept.	81	76	347	—	4.5-6.0-6.8	3.7-5.7-6.6	12+ — —

*Exposed to wind and indirect sunlight.

A similar number of flies was kept in test-tubes for control purposes, and the time of survival of each fly was recorded to the nearest tenth of an hour. After contact with the insecticide, the flies showed the usual train of symptoms of DDT-poisoning. It was noticed, however, that the interval between contact with DDT and collapse was by no means proportional to the total survival time. There was great variation in the degree of restlessness of the flies, particularly after paralysis of the legs set in. Some, while lying on their backs, apparently otherwise helpless, would maintain violent intermittent wing vibration for hours, but sometimes, when a fly, lying deceptively quiescent after a spell of activity, was taken out of the test-tube for closer examination, it would escape through the window in seemingly normal flight. How prolonged the life of such a poisoned fly is likely to be is not known. The tsetse were under constant observation for 12 hours in all the experiments and no individual was seen to develop symptoms of DDT poisoning and subsequently to recover as has been recorded in the case of mosquitos (Kennedy, 1947). On the other hand, frequently a fly seemed to be resisting the effects of contact with DDT and the onset of visible toxic symptoms was delayed, yet the eventual collapse would be very quickly followed by death. The final symptoms of DDT poisoning in tsetse were spasmodic movements of the proboscis which were not observed to occur until wing and leg movements had almost or completely ceased. They could be taken as a sign that death would take place within a few minutes. In death the mouthparts were invariably gaping widely.

Results.

Table I summarises the tests. The survival of a control fly is measured from the time of its enclosure in a test-tube, that of an exposed fly from the time of contact with DDT. With a few exceptions each assessment is based on the observations for 20 or 24 flies; when fewer flies were used it was because they were unobtainable.

The completion of the practical work by midday left 12 hours for observation and the majority of the flies exposed to DDT died well within this limit. If, however, a fly did not die within twelve hours it might succumb during the night, in which case its survival was some unknown interval between 12 and 19 hours; or it might survive 24 or 48 hours or even more, and its death was almost certainly not due to DDT-poisoning. In the case of the control flies, the survival times might be anything from a few hours to a few days. The experiments were carried out at the prevailing shade temperatures and observation of the control flies showed that, if it was restless in the heat of the afternoon, an individual was liable to wear itself out and die before night, but that if it remained fairly quiet it would probably be in good condition the next morning. Also, any tsetse which survived for a whole day might survive for two or even three days. It was obvious that, with the experimental flies, the atypically long survivals would destroy the value of an average based on the arithmetic mean and to avoid their unbalancing effect the median was chosen as the most typical representation of the average survival with the lower and upper quartiles as a measure of the variation.

The results show that, after 20 weeks' exposure to the weather, the impregnated cloth still retained an appreciable toxicity but that by 30 weeks this effect was very greatly reduced. At a final test made after 50 weeks' exposure, which included the wettest months of 1947, the toxicity was negligible and was manifest only in flies which had had a 60-second contact. On the other hand, the cloth which had been exposed to wind and daylight but sheltered from direct sunlight and rain could still, at the end of 50 weeks, produce definite toxic symptoms as the result of only 15 seconds' contact.

The next significant fact is that, with the exception of the results for 14th and 22nd October, there is very little difference between the effect of 15-second and 60-second contacts with DDT. From the sum of the results of the first 20 weeks, when

the cloth was still highly toxic, 7.5 per cent. of the flies survived longer than 12 hours after a 15-second contact, but after a 60-second contact only 4 per cent. survived longer than 12 hours. Apart from these differences, and the fact that on any one day the first death was usually from a 60-second contact, the general ranges of survival times from corresponding tests were quite similar. In view of the proposed application of DDT to tsetse traps, this is a very important result. It shows that if DDT is present in sufficient strength and availability to poison a tsetse coming in contact with it, then a brief contact only will suffice, and a more prolonged contact is not likely to influence the final outcome.

In each test there was a great diversity among the survival times which must have resulted from the inherent resistance of the flies. Observation suggested size as a factor but this could not be investigated quantitatively. Analysis of all the results showed that the average survival of females was slightly longer than that of males; females also tend to be slightly larger than males. Additional tests with *G. palpalis* showed that on the whole it had a longer survival than *G. tachinoides* but probably only in so far as its average size was greater.

Possible reasons for the failure of some flies to develop symptoms of poisoning after supposed contact with DDT are: uneven distribution of the DDT through the cloth; occlusion of the DDT by particles of dirt adhering to the cloth; fortuitous protection resulting from a film of some foreign matter on the tarsi acquired in a previous environment. The fact that the longer contact resulted in fewer of these escapes favours the first two hypotheses. In later experiments, hessian was impregnated with two different strengths, one portion of each being exposed to the weather, another being kept in an airtight box as a control. In the case of the heavily impregnated control cloth, not a single fly in all the tests escaped death with symptoms of poisoning, but 3 per cent. survived longer than 12 hours (and may have died from other causes) after contact with the exposed piece which, nevertheless, retained a very high degree of toxicity after 6 months. The corresponding figures for the protected and exposed pieces of lightly impregnated hessian were respectively 4 and 15 per cent.

It is important to note that the survival times of both the control flies and those exposed to DDT change with the progress of the season. It is not surprising that higher temperatures and reduced relative humidities lower the expectation of life of the control flies in the unnatural habitat of a test-tube, but what was unexpected was the spectacular shortening of the survival times of the experimental flies towards the end of October and throughout November and December. During the first fortnight of the tests, the survival times of the test flies were already lengthening appreciably and the impression was formed that the persistence of toxicity, if any, would soon be so slight as to be of little practical importance. This was in agreement with the findings of Hocking, Hadaway and Woodcock of the Colonial Insecticide Research Station, Entebbe. These workers sprayed cotton twill and hessian with a 5 per cent. diesel oil solution of DDT and then exposed it to the weather. Basing their results on the numbers of flies dead 6 hours after a 15-second contact with the cloth, they found that mortality fell from 100 per cent. on the newly prepared hessian to 40 per cent. after 14 days and to only 15 per cent. after 24 days; the cotton twill was slightly more efficient. They concluded that for an adequate degree of toxicity to be maintained the cloth must be resprayed at weekly intervals, and that four such applications gave a cumulative effect which would last for a further 3-4 weeks. At Lawra, it was decided to continue the experiments so as to obtain information which might be of academic if not of practical interest, and this revealed what appears to be the heightened susceptibility of the tsetse to DDT poisoning under hot dry conditions.

In the first 40 days the impregnated hessian was rained on 11 times and this presumably washed away some of the DDT. During the whole period it was exposed

to wind and strong sunlight. According to Gahan and others (1945), sunlight has a deleterious effect on deposits of DDT and, if treated boxes and cages are kept in complete darkness, in the shade of the laboratory and in full sunlight (but protected from rain), longer exposures are required to knock down mosquitos in the containers kept out of doors than in any of the others. The boxes, which had been kept in the shade and in darkness, were still able to cause 100 per cent. mortality (24 hours after contact) 40 weeks after treatment but the box subjected to sunlight was completely effective for only 24 weeks. The effect of wind would also be expected to reduce the amount of DDT present by loosening it and blowing it away, or to reduce the amount available by blowing dust on to the hessian. Thus by November, and still more so by December and January, the impregnated hessian must have had a lowered DDT content despite the fact that the biological tests suggested an increased efficiency. By means of various statistical treatments of the data, attempts were made to obtain a measure of the loss of efficiency of the hessian but this was not found to be possible; even the mere demonstration, without measurement, of loss of efficiency was not entirely satisfactory. Table II summarises two of the methods used.

TABLE II.

Two methods of estimating mortality in *G. tachinoides* after contact with DDT-impregnated hessian.

Date	Exposure of cloth to weather	% Tsetse Dead 6 hours after contact with DDT	% Controls Dead in 6 hours	% Mortality in Test flies (corrected to allow for mortality in Controls)	Time required for death of 10% of Controls hrs.	% Mortality of Test flies at this time
<i>Hessian fully exposed to weather</i>						
1946						
2-3 Oct.	1-2 days	74	0	74	10.5	95
7 "	1 week	67	0	67	12	93
14-15 "	2 weeks	51	0	51	approx. 12	85
22-23 "	3 "	60	6	57	approx. 7.1	69
28 "	4 "	64	14	58	5.2	40
7 Nov.	5½ "	70	15	65	5.3	66
23 "	8 "	96	56	91	2.8	50
7 Dec.	10 "	95	37	92	4.2	72
21 "	12 "	98	62	95	2.6	54
1947						
18 Jan.	16 "	98	40	97	3.5	60
21 Feb.	20 "	73	17	67	5.5	67
28 Apr.	30 "	21	17	5	4.7	17
12 Sept.	50 "	4	0	4	11.3	38
<i>Hessian protected in veranda</i>						
1947						
28 Apr.	30 "	71	17	65	4.7	46
12 Sept.	50 "	60	0	60	11.3	96

In order to give numbers suitable for the calculation of percentages, the results of tests carried out on consecutive days are grouped and the survival times of the 15-second and 60-second contacts are considered to be homogeneous. The first method of calculation adopts the commonly used convention of taking the proportion of flies dead 6 hours after contact with DDT. In a test made with a few flies on the day the cloth was prepared, and while it was still obviously permeated with kerosene,

the flies were all dead within 6 hours. On this basis, the conclusion must be that the cloth was reduced in efficiency by 25 per cent. in 2 days and by 50 per cent. in 2 weeks, after which it began to improve until, after a further 6 weeks, it was almost as good as new and remained so for another 8 weeks.

Parkin and Green (1947) worked on the persistence and toxicity of deposits of DDT in kerosene solution sprayed on wall-board. They recorded the percentage knock-down of house-flies at the end of various periods of exposure ($\frac{1}{2}$ hour-4 hours) to the DDT films, and found that with certain combinations of concentration and deposit the toxicity was definitely greater at four weeks than on the fourth day. After the fourth week there was a decrease in toxicity followed in many cases by a second increase, usually between the twelfth and twentieth weeks. It appeared that this increase was "due to crystallisation of DDT from the supersaturated solution left after evaporation of the volatile constituents of the spray", and that the crystallisation might be stimulated by "any form of mechanised agitation, including movement of the flies on the treated surface." The main difference between these results and those of the tsetse experiments is that in the latter case the initial change in toxicity was always a drop. Moreover, the sprayed wall-boards were maintained at a constant temperature and humidity whereas the hessian was exposed to the natural daily variations of temperature and to the changes due to the onset of the dry season. The effect of the dry season, with its increased temperatures and lowered humidities, is seen as a rise in the percentage mortality of the controls recorded after 6 hours, and this should be taken into account. The fifth column of Table II gives an estimated percentage mortality of the experimental flies on the assumption that the factors causing death from DDT poisoning and those leading to death from other causes operate independently of one another. Suppose that the proportion of flies that would have died from DDT poisoning alone is "a", that the proportion that would have died simply from being confined in test-tubes is "b" (mortality of controls) and that the proportion that actually died from the combined effect of contact with DDT and being in a test-tube is "c". Then the proportion still alive, $1-c$, is also $(1-b)(1-a)$, giving $a = \frac{c-b}{1-b}$. This adjustment is seen to make

little difference to the results which may therefore seem to be in close agreement with the findings of Parkin and Green. It is considered, however, that the underlying cause is different in the two cases. The surfaces of the wall-boards were observed to regenerate a crystal carpet of DDT but all other factors remained constant. The surface film on the hessian may well have changed with the wear and tear of the weather but the effect of the weather on the tsetse is likely to have been of much greater importance.

The second method of estimating loss of efficiency is based on the effect of the changing season on the mortality of the controls. The mortality of the experimental flies is measured not in relation to an arbitrarily chosen fixed interval of time after contact with DDT but in relation to the time by which the death had occurred of a definite proportion of the controls. Ideally, the choice might be the survival time of the first control to die since there could then be no question as to the cause of death of experimental flies whose survival times were less than this. But in practice the most careful selection of flies sometimes included weakly specimens in which death was premature, even for the unnatural environment of the test-tube. In order to avoid the prejudicial effect of these exceptions, it is better to make the interval selected more representative of all the controls and in this case 10 per cent. mortality of the controls has been used giving survival times that vary from 12 hours in October to 2.6 hours in December. On this basis the mortality curve of the flies exposed to the impregnated hessian is now quite different. The drop during October from 95 to 40 per cent. is followed by a fluctuating rise which may or may not be significant—it depends on whether the interval of 5.2 hours for 28th October

happens to be rather low. If it is too low then the results suggest that the hessian loses some of its toxicity in the first fortnight or so and that it can then be expected to maintain a reduced but fairly reliable degree of efficiency over a period of fully four months of dry season conditions. The figure of only 17 per cent. mortality after 30 weeks' exposure followed by one of 38 per cent. mortality after 50 weeks' exposure is an unfortunate discrepancy. That a similar inconsistency appears in the corresponding mortalities from the protected hessian indicates that the survival interval of 4.7 hours, as derived from the controls, is too short.

The great difference in the results obtained by the two methods make it clear that at least one of them must be giving a misleading picture. It is probable that the more orthodox method could not make adequate allowance for a possible increase in speed of reaction to DDT poisoning at higher temperatures or reduced humidities, nor distinguish between change of speed of reaction and decreased efficiency of the hessian. As regards efficiency it obviously makes little difference whether all the flies that have been in contact with DDT die within four hours or six hours provided that they all die with typical symptoms. In September, 1947, it was decided to repeat the experiment (see page 269), measuring progressive change of toxicity of the exposed hessian against the toxicity of a control piece of the same cloth protected from light and air.

The Effect of Moisture on Toxicity.

The effect of moisture on the toxicity of DDT-impregnated hessian was investigated. This is important, for, whilst it was evident that exposure to a considerable amount of rain had not washed away the DDT to any great extent, there was the possibility that the presence of moisture in the form of rain-water or dew might have a temporary influence on the potency of the impregnated cloth. Table III summarises the results of the tests.

The hessian used on 30th October had been prepared two days previously and there was still an odour of kerosene about it when the first experiment was carried out. Thirty tsetse were given 30-second contacts with the cloth in the ordinary way and then a representative area was moistened by the application of a small quantity of distilled water from a pipette and 30 more flies were exposed to it. Drops of water were added from time to time so that the cloth felt damp to the touch for as long as was required. Since water cannot be used as a solvent for DDT, the result of this test, showing the moistened hessian to be more toxic than the dry, was unexpected. The same piece of hessian was used a week later, the kerosene having by then completely evaporated. After 30-second contacts had been made with 25 flies on the dry cloth and with 25 on an area moistened with water, a further batch of 25 was exposed on another area moistened with a few drops of kerosene. This time the effect of the water was to reduce the apparent strength of the DDT while the kerosene very markedly increased it. Repetitions on 2nd and 8th December gave exactly similar results. Included for comparison with the results of 8th December are the survival times in test-tubes of unexposed control flies and of flies which had been given 30-second contacts with clean hessian moistened with kerosene. The flies exposed to kerosene alone die off much more rapidly than the control flies. The fact that the toxicity of DDT-impregnated cloth is increased when it is moistened with kerosene may thus be due either to the sum of the independent toxic effects of the DDT and the kerosene or, possibly, to their joint effect, the integument of the tarsi being more readily penetrated by the DDT when in a kerosene solution. The presence of traces of kerosene in freshly treated hessian and its subsequent complete evaporation may also explain the great difference between the results of the first day's experiments on a piece of test cloth and those of the second and third days, as illustrated in Tables I and V. To judge from the survival times of the exposed flies there is an immediate

drop in the toxicity of the cloth which may give a misleading impression that such a method of utilising DDT is not very practical. Presumably what really happens is the evaporation of the last of the kerosene and not a deterioration of the DDT content of the cloth.

TABLE III.

The effect of the presence of moisture on the survival times of *G. tachinoides* after 30-second contacts with DDT-impregnated hessian.

		Summary of Survival Times in Hours			
		Average	Interquartile Range and Median Values		
The same piece of impregnated hessian was used in all these tests. Tsetse were exposed first to dry cloth which was afterwards moistened	30 October				
	Dry	4.7	3.0	4.7	6.4
	Wet	3.8	2.4	3.0	5.7
	6 November				
	Dry	4.7	3.2	4.8	6.0
	Wet	5.8	3.4	5.3	8.7
	With kerosene ...	2.9	2.4	2.8	3.6
	2 December				
	Dry	3.9	3.1	3.6	4.7
	Wet	4.2	3.1	3.8	5.2
	With kerosene ...	3.1	2.4	2.8	4.3
	8 December				
	Dry	4.3	3.1	4.0	5.0
	Wet	5.7	3.7	6.1	7.7
	With kerosene ...	3.1	1.9	2.5	3.6
	Kerosene alone ...	6.9	3.5	6.5	9.1
	Unexposed controls	14.4 approx.	7.8	15 approx.	19.5
	9 December				
	Wet	5.2	3.2	4.8	6.0
	Damp	4.1	2.8	3.3	4.3
	Dry or nearly dry	4.3	2.6	3.6	5.3
New piece of impregnated hessian with slight smell of kerosene	5 January				
	Dry	3.1	2.4	3.2	4.0
	Wet	3.7	3.0	3.7	4.5
	Drying	3.8	2.9	3.5	4.6
	6 January				
	Dry	3.8	2.3	4.0	4.4
	Wet	3.6	2.6	3.8	4.2
	Drying	—	4.8	7.1	15 approx.
	7 January				
	Dry	4.4	3.3	4.6	5.4
	Damp and drying	—	5.0	8.4	15 approx.

On 9th December and 5th January the same piece of cloth was used and on both occasions proved less efficient wet than dry. On 6th January a fresh piece was used. This was cloth which had been impregnated in September and stored in a closed jar. It still retained a slight smell of kerosene and, as before, the experimental flies

succumbed more quickly when the cloth was wet than when it was dry. The conclusion is that although normally the presence of water interferes with the insecticidal action of DDT, a little water is not detrimental and may even be an advantage in the presence of residual traces of the kerosene solvent. It will be noticed that for the 6th and 7th January results are given for exposures to "drying" cloth. The cloth in these cases was only slightly moist at the time of the tests and it was expected that its efficiency would differ very little from that of cloth in the dry state. But since one-fourth of the exposed flies survived longer than 12 hours and were not seen to develop toxic symptoms something in the physical state of the cloth must have prevented proper contact of the flies with the DDT, or otherwise inhibited its action. Possibly atmospheric conditions gave rise to similar phenomena in the hessian exposed to the weather, so causing some of the irregular results to be seen in the September and October entries of Tables I and V.

Hessian treated with emulsifiable DDT.

Another test, which gave promise of useful results, was carried out with DDT in the liquid, emulsifiable form. Jeyes' "Atso", containing 18 per cent. DDT, 58 per cent. naphtha and 24 per cent. emulsifier, was used and pieces of hessian were impregnated in three ways: by soaking in the neat liquid, by soaking in emulsions made from 50 per cent. DDT with 50 per cent. water and from 25 per cent. DDT with 75 per cent. water. The pieces of hessian were cut into two lengthways as soon as they were dry, one half being hung outside on a tree and the other kept in a box. Table IV summarises the results of 30-second contacts made with batches of 25 flies, in the first instance when the samples were freshly prepared, and again 10 to 12 weeks later.

TABLE IV.

Summary of results of tests for persistence of toxicity in hessian impregnated with emulsifiable DDT Samples prepared on 19.xii.47.

				Survival in hours of batches of 25 <i>G. tachinoides</i> after 30-second contacts with impregnated hessian.			
				Average	Interquartile Range and Median Values		
December 22 and 23.							
Neat DDT	5.4	3.4	4.8	6.5
50% Emulsion	3.3	2.3	3.1	3.8
25% Emulsion	3.6	2.6	3.1	4.0
Control tsetse in test-tubes	...			—	8.8	12+	—
March 4. Neat DDT.							
Exposed on tree 11 weeks	...			6.2	3.5	5.4	9.0
Control from box	6.0	4.0	4.8	8.0
Control tsetse in test-tubes	...			—	7.0	8.9	12+
March 2. 50% Emulsion.							
Exposed on tree 10½ weeks	...			4.9	3.0	4.7	6.5
Control from box	4.9	4.0	4.7	5.7
Control tsetse in test-tubes	...			—	6.5	8.9	10.2
March 11. 25% Emulsion.							
Exposed on tree 12 weeks...	...			4.6	3.3	4.1	5.6
Control from box	4.7	2.9	3.9	6.7
Control tsetse in test-tubes	...			—	6.7	8.9	11.5

Both strengths of emulsion were much more effective than the neat liquid and, after nearly three months' exposure, the two emulsion samples appeared to have lost no efficiency, and the neat DDT sample only a slight proportion, compared with the corresponding protected samples. The weather during the period of exposure was hot during the day, cooler at night, with dusty, very drying winds and no rain. It

should be noted that the survival times were on the whole much shorter in December than in March. This experiment unfortunately could not be continued on account of the writer's departure on leave, but it would be useful to know how long this toxicity could be successfully retained and, above all, whether cloth impregnated with an emulsion of DDT would stand up to the effects of rain. Information on the success and relative toxicity of weaker emulsions would also be of value.

The Measurement of residual Toxicity in Hessian.

The new cloth used for this experiment was washed to remove dressing, dried, and a piece four square feet was soaked for ten minutes in a half-gallon of solution prepared as described on page 260. Cloth prepared in this way will be referred to as standard impregnated hessian or more briefly, standard cloth. On the following day one square foot of the standard cloth was returned to the kerosene solution and soaked for an hour. The effect of this was to cause a deposition of fine crystals on the fibres of the hessian that, when dry, gave a much whiter appearance to it than to the standard cloth. This will be known as super-impregnated hessian or super cloth. Subsequent analysis made at the Colonial Insecticides Research Laboratory, Porton, England, showed that the standard and super cloths received respectively 4.4 g. and 7.4 g. of DDT per square foot. As before pieces of each were fully exposed to the weather; in addition, samples were kept as controls, the super cloth in an airtight box, the standard folded in paper.

In all the tests, the tsetse were given 30-second contacts with the impregnated hessian. For every batch of flies, 25 when possible, with which either the standard or the super exposed cloth was tested, the corresponding unexposed control sample

TABLE V.

Summary of the results of experiments to determine survival times of *G. tachinodes* after 30-second contacts with hessian impregnated with DDT in two strengths.

Standard Hessian

Date	Exposure of Cloth		Test Sample		Control Sample	
	Time	Rain	No. of Tsetse	Survival in Hours. Interquartile Range and Median	No. of Tsetse	Survival in Hours. Interquartile Range and Median
September	Days					
15-16	1-2	.05 ins. on 1 occasion	27	2.8 3.4 5.4	27	2.7 3.3 4.4
18 & 23	4-9	2.08 " 5 occasions	29	3.1 5.1 6.2	29	2.4 4.1 5.4
25-26	11-12	2.44 " 7 "	25	5.3 6.7 8.3	25	3.6 4.2 5.3
29-30	15-16	4.37 " 9 "	27	6.0 7.9 15	27	3.1 4.4 5.4
October	Weeks					
6-10	3	5.20 " 11 "	25	4.8 9.8 15	25	3.3 5.0 7.3
13 & 16	4	6.16 " 14 "	28	7.2 15 21.8	27	3.5 5.9 15
28	6	6.22 " 15 "	25	3.8 5.6 9.5	25	3.1 4.0 6.9
Nov. 4	7	6.22 " 15 "	22	3.2 5.4 7.9	22	3.1 4.5 6.8
December						
1-2	11	6.22 " 15 "	25	3.9 4.9 5.9	25	3.6 4.6 5.8
15	13	7.42 " 16 "	25	3.1 4.1 5.8	25	2.6 3.3 5.2
30	15	7.42 " 16 "	25	3.5 4.2 5.2	25	2.5 4.0 5.5
January						
12	17	7.42 " 16 "	25	2.9 5.2 8.2	25	3.7 4.7 6.1
March						
1	24	7.42 " 16 "	25	4.0 5.3 7.2	25	4.2 4.9 6.6

Table V —continued.

Super-impregnated Hessian

Exposure of Cloth		Test Sample		Control Sample:	
Date	Time	Rain	No. of Tsetse	Survival in Hours. Interquartile Range and Median	No. of Tsetse. Survival in Hours. Interquartile Range and Median
September	Days				
16-17	1-2	·15 ins. on 2 occasions	21	2·2 2·9 3·7	21 1·6 2·2 2·8
19 & 24	4-9	2·20 „ 6 „	27	2·8 3·9 5·2	27 2·6 3·4 4·5
25-26	10-11	2·44 „ 7 „	25	3·2 4·1 5·4	25 2·7 3·2 3·8
29-30	14-15	4·37 „ 9 „	27	3·6 4·6 6·5	27 2·5 3·2 4·1
October	Weeks				
6-10	3	5·20 „ 11 „	25	4·5 6·2 7·6	25 2·5 3·1 4·6
13 & 16	4	6·16 „ 14 „	28	3·6 4·8 6·1	28 2·1 2·8 3·7
23	5	6·22 „ 15 „	24	3·3 3·6 5·1	25 2·1 3·0 3·7
30	6	6·22 „ 15 „	25	3·3 4·1 5·7	25 2·8 3·2 4·2
November					
6	7	6·22 „ 15 „	25	2·4 3·6 4·6	25 2·4 3·1 3·7
December					
1	11	6·22 „ 15 „	25	2·6 2·9 3·6	25 2·1 3·0 3·4
15	13	7·42 „ 16 „	25	2·8 3·4 3·8	25 2·1 2·8 3·6
29-30	15	7·42 „ 16 „	25	2·7 3·3 4·4	25 2·2 2·9 3·6
March					
1-2	24	7·42 „ 16 „	52	4·1 5·1 6·2	52 2·7 3·7 4·7
11 & 14	26	7·42 „ 16 „	47	2·9 4·2 5·2	48 2·3 2·8 3·7

True Controls—Tsetse kept in test-tubes.

Date	No. of Tsetse	Survival in Hours. Interquartile Range and Median		
September				
12-19	67	15	32	49
23-24	33	15	29	45
27	51	13	23·5	42
October				
8-11	47	14	21	33
16	21	15	26	31·5
23 & 25	64	15	24	30
31	30	6·9	15	21
November				
6 & 8	62	11·3	15	18·3
December				
1-2	39	6·7	9·5	15
8-9	64	7·5	15	19·2
15	21	10·3	15	21·2
29	28	6·3	15	18
				approx.
January				
13	26	7·8	15	19·5
March				
2-3	63	6·7	8·9	11·5

In each case a sample of the cloth which had been exposed to the weather was tested against a protected control sample, and, in addition, a number of flies was kept as true or unexposed controls to give a measure of survival under the conditions of the experiment, *viz.*, confinement in test-tubes at the prevailing temperatures.

was tested with a batch of the same number. The control samples were handled as little as possible, the super cloth being kept in its box, but even the application of the rim of the test-tubes had its effect and the crystals on the super cloth gradually became reduced to a dense fine powder, a change which took place much more quickly on the exposed sample. By the end of six months the DDT was no longer apparent on the exposed samples, although the super cloth was still distinguishable from the standard cloth. The control samples, apart from the breaking down of the crystals, appeared to be unchanged; they were still obviously well charged with insecticide.

The new experiments confirmed the previous series in showing that prolonged outdoor exposure did not reduce the toxicity of DDT-impregnated hessian below the limits of usefulness. In addition, they provided data from which toxicity could

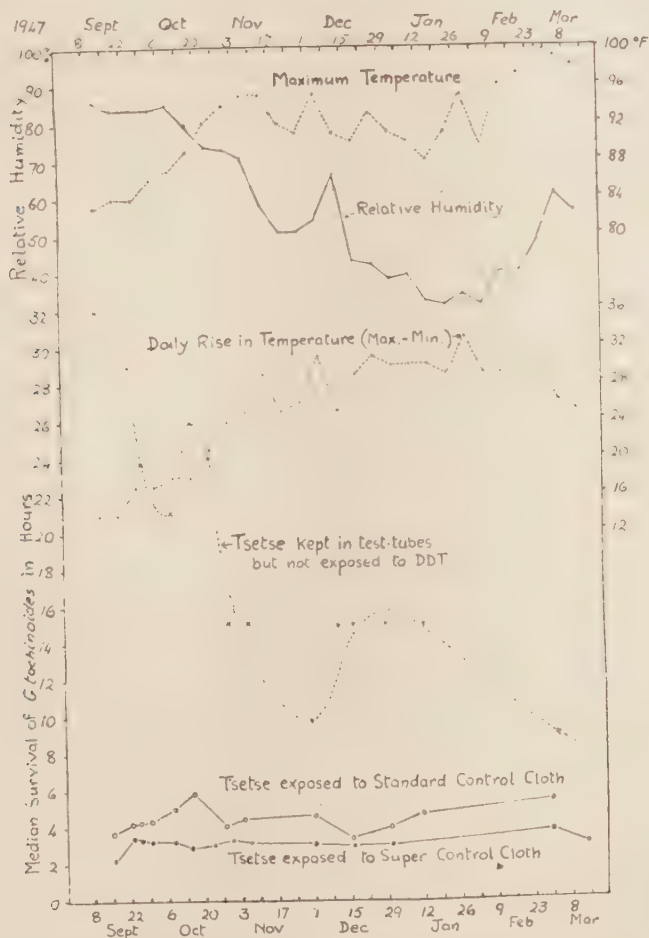


Fig. 1.—The changes in weather conditions during the six months' test for the persistence of toxicity in DDT-impregnated hessian and their effect on the survival of *G. tachinoides* in test-tubes, with and without exposure to DDT.

be estimated in various ways and the advantages and drawbacks of each method could be assessed. Table V summarises the results. Flies were scarce in September and early October and in order to have a minimum of 25 flies in each category certain of the observations are grouped. It invariably happened that many of the true controls died during the first night after the start of any particular experiment and these are all said to have survived 15 hours. This arbitrary value, which may have been anything from 12.5 hours to 18.5 hours, therefore appears frequently in the table and graph of true control results.

Fig. 1 gives the weekly mean maximum temperatures and relative humidities (from 8 a.m. readings on a whirling psychrometer) for the six months of the experiments; it also shows the weekly mean of the daily rise in temperature, *i.e.*, the difference between the maximum and the minimum of the previous night. These changing weather conditions are related to the median survival times of flies in test-tubes, with and without exposure to DDT. The graphs suggest that the survival of the true controls is to some extent inversely proportional to the maximum temperature. The poor survival in March when the relative humidity had risen a little was probably due to the flies being on the whole smaller than they were in November. The increased relative humidity for the period 8–15th December resulted from a heavy and unusually late rain (1.20 inches on 10th December) and very likely this rain was responsible for the better survival of the flies in the late December and January controls compared with those of the same period in the previous year.

The scale on which fig. 1 is drawn serves to emphasise the consistency of the results from the control sample of super-impregnated hessian. The initial rise is considered to be due to the loss of the additional toxicity resulting from the presence of traces of kerosene. The weaker preparation of DDT gave more variable results. The peak for 13th–16th October corresponds to that in the true control and is repeated in the result from the exposed sample of the same cloth—a median survival of 15 hours which gave the impression at the time that useful toxicity was already completely lost, yet this exposed cloth proved to be quite efficient later. One explanation of this apparent lapse is that certain atmospheric conditions inhibit

TABLE VI.

Survival of male and female *G. tachinoides* after 30-second contacts with super-impregnated hessian.

Date	Males		Females		All Tsetse		Percentage of Females in Flyboys' Catches
	No. of Tsetse	Average Survival	No. of Tsetse	Average Survival	Average Survival	Corrected Average	
September 15–30	57	Hours 3.2	63	Hours 3.2	Hours 3.2	Hours 3.5	58
October 6–16	22	2.8	30	3.8	3.4	3.6	57
Oct. 23—							
Nov. 6	32	2.5	40	3.7	3.2	3.4	52
December 1–30	47	2.5	27	3.4	2.9	3.1	40
March 1–14	33	3.5	66	3.5	3.5	3.7	67
For all tsetse:		2.93		3.45			

the action of DDT so that the toxic effect is reduced or delayed. Comparison of the day to day differences between experimental results showed that high humidity was associated with lengthened survival times from all the samples except the super control in its airtight box.

The survival times from all four samples of impregnated cloth became lowered in December, a time when conditions, although not most adverse for the true controls, included a combination of low humidity with high daily change in temperature. One possible cause of this reduced survival was the differential effect of DDT poisoning on the sexes, since in December the percentage of males in the available flies had become higher. Table VI, based on the results of all the super cloth tests, shows that the survivals of both males and females became reduced in December and that the females tended to survive one-sixth as long again as the males. Even if this correction is applied to the high proportion of males in the December results, the average survival for that period is still exceptionally low. The changed sex ratio can thus only partially account for the higher mortality; the smaller size of the flies is probably a more important factor. From all the information available it is quite evident that, in the case of tsetse, susceptibility to DDT poisoning varies according to seasonal conditions. When they have been in contact with DDT in high concentration, the average survival of tsetse in a test-tube is about three hours, individual cases varying from one hour, or occasionally less, to about six hours. The onset of conditions such as those in December reduces the maximum survivals to 4-5 hours but has only a slightly lowering effect on the average survival. When a weaker concentration of DDT is used, allowing initially much longer survivals to the flies which have touched it, December conditions caused a much more noticeable shortening of the survival times so that they become more like those from the high concentration. These effects are all the more spectacular when they result from samples of impregnated cloth which have been exposed to the weather and which are therefore presumed to have lost some of their DDT content.

Comparison of "standard" and "super" control samples of hessian.

As a preliminary step to the assessment of loss of efficiency in the exposed hessian, the difference between the results from the standard and super-impregnated samples was determined. For this the results of the tests on the control samples up to the end of December were used, omitting the initial tests made when the presence of kerosene was increasing the toxicity of the cloth: the later tests were not comparable as regards the numbers of flies used on the two strengths of DDT. For convenience the data are presented in summary in Table VII grouped into five successive periods each including numbers of observations large enough to be used for the calculation of percentages. These periods, 17-30th September, 6-16th October, 28th October-4th November, 1st-30th December, 1st-14th March will be referred to as September, October, November, etc. The data are arranged to show the times, after contact with DDT, for the death of 10 per cent., 20 per cent., etc., of the experimental flies; the corresponding mortalities of the true controls up to 12 hours are included. As changes in the size of the tsetse and in their sex ratio were the same for each series these factors need not be considered.

TABLE VIII.

Various methods of recording and expressing the mortality of *G. tachinoides* after 30-second contacts with "standard" and "super" control cloth.

	Sept.	Oct.	Nov.	Dec.	Efficiency of Standard cloth in relation to Super cloth.			
					Sept.	Oct.	Nov.	Dec.
					Relative mortality of flies			
I.—Percentage of tsetse dead 6 hours after contact with DDT					%	%	%	%
Super	97	98	97	100				
Standard	83	58	72	85	86	59	74	84
Percentage of controls dead in 6 hours	3	1	13	13				
Mortality of test tsetse corrected to allow for mortality in controls								
Super	97	98	97	100				
Standard	83	58	68	83	86	59	70	83
II.—Time required for death of 100% of tsetse exposed to super cloth	8.3 hs.	6.3 hs.	8.4 hs.	5.3 hs.				
% tsetse from standard cloth dead in this period	96	58	87	76	96	58	87	76
Time required for death of 75% of tsetse from super cloth ...	4.1 hs.	4.0 hs.	3.7 hs.	3.5 hs.				
% tsetse from standard cloth dead in this period	48	35	47	43	64	47	63	57
Time required for death of 50% of tsetse from super cloth ...	3.2 hs.	3.0 hs.	3.1 hs.	2.9 hs.				
% tsetse from standard cloth dead in this period	25	19	30	24	50	38	60	48
III.—Time when first control died... % exposed tsetse dead in this period	4.0 hs.	4.2 hs.	3.1 hs.	3.0 hs.				
Super	74	75	52	63				
Standard	44	40	30	27	59	53	58	43
Time required for death of 5% of controls	7.7 hs.	7.1 hs.	4.7 hs.	4.5 hs.				
% exposed tsetse dead in this period								
Super	99	100	97	97				
Standard	91	63	62	61	92	63	64	63
Time required for death of 10% of controls	9.0 hs.	8.8 hs.	5.8 hs.	5.5 hs.				
% exposed tsetse dead in this period								
Super	100	100	97	100				
Standard	98	73	70	79	98	73	72	79
IV.—Estimate of efficiency of Standard cloth in terms of Super cloth based on median survival times thus:— True Control—Standard								
True Control—Super					96	90	91	90
V.—Time required for death of 100% of tsetse	Hours	Hours	Hours	Hours	Relative time required for DDT to take effect			
Super	8.3	6.3	8.4	5.3				
Standard	10.0	29.6	48	15	1.20	4.70	5.71	2.83
Time required for death of 75% of tsetse								
Super	4.1	4.0	3.7	3.5				
Standard	5.4	9.5	6.8	5.3	1.32	2.37	1.84	1.51
Time required for death of 50% of tsetse								
Super	3.2	3.0	3.1	2.9				
Standard	4.2	5.1	4.2	4.1	1.31	1.70	1.35	1.41

It was known that 30 seconds' contact with the heavily impregnated cloth was sufficient to kill every tsetse. The intervals between contact and death varied from less than one hour to more than eight hours but, since in all cases the flies died with the same type of symptoms, the whole range and distribution of the survival times represent 100 per cent. efficiency. The weaker preparation was obviously less effective. The survival times, although ranging as low as those from the super cloth, are not only longer on the whole but include some of 15 hours and more that are at once suspect. It is, therefore, logical to suppose that as the standard cloth had a DDT content which was a definite fraction, 0.59, of that of the super cloth, so also would its efficiency be in a measurable proportion. The ratio of efficiency is not necessarily the same as that of DDT contents because the super cloth, bristling with DDT crystals, might have given equally good results if slightly less had been present, but at least it must be finite. A precise knowledge of this relationship is necessary for an understanding of the progressive decrease in efficiency of the exposed samples of cloth.

The results of several methods of calculating the toxicity of the standard and super control samples of hessian and of expressing the efficiency of the former in terms of the latter are given in Table VIII. The most generally used criterion is the percentage of flies dead within some specified interval of time after the contact of the flies with DDT. If it is considered that the result achieved by the super cloth is the maximum possible for killing tsetse by means of a DDT impregnation then the efficiency of the standard cloth is

$$\frac{\text{Mortality from Standard Cloth}}{\text{Mortality from Super Cloth}} \times 100. \quad \text{Three}$$

different ways of selecting the interval are illustrated. Each of these appears reasonable but, from the diversity of the efficiency ratios they give rise to, it follows that some or all are not reliable. The most that they reveal is a relative ineffectiveness of the weaker preparation under the conditions prevailing in October. The great variations resulting from the adoption of Methods II and III should be noticed. These suggested that the difference between the two types of cloth might be one of quickness or slowness of the induced reactions in the flies rather than that of absolute killing power. Method IV shows that concept, by itself, to be equally unsatisfactory in producing a definite result.

In the final estimate of the relative efficiency of the standard cloth, the survival times of the flies from the super cloth are taken to represent 100 per cent. efficiency and those of the control flies zero efficiency. Suppose that the median values of the survival times for the super cloth, the standard cloth and the unexposed flies are 3, 5 and 13 hours respectively. The flies from the super cloth, represented by their median, die ten hours sooner than the control flies, but those from the standard cloth die only eight hours sooner than the controls. The standard cloth is therefore 80 per cent. as efficient as the super cloth. This method of calculation gives a more constant value than any of the others for the efficiency in the different periods, but it is one which should really be applied to the arithmetic means of the survival times, an average which in the case of the unexposed flies cannot be accurately calculated.

These preliminary calculations involve two different principles. In one, a particular interval of time is chosen and mortalities within that period are compared; in the other, a particular percentage of mortality is chosen and the times necessary to achieve this are compared. Neither of these is sufficient by itself but a solution was provided by the fact that the slower reaction of a fly to DDT does not necessarily mean that it has been less successfully killed than another which dies more quickly because the DDT is stronger. The important point is that the experimental fly in the test-tube should die with symptoms of poisoning. The essential efficiency of a particular piece of impregnated hessian is the proportion of flies that its contact is capable of killing, for the fly which dies merely of exhaustion in the test-tube

corresponds to one which would escape if the hessian were put to practical use. The time that a fly takes to die after being poisoned may be of theoretical interest but is not likely to be of practical importance, for the probability that any particular fly is likely to transmit trypanosomiasis after fatal contact with DDT must be so infinitesimal that the difference in this probability, as affected by an additional hour or so of survival would be negligible. The real difference between the super and standard cloths must be expressed in terms of both the relative proportion of the flies killed and their relative survival times.

The method of finding the relative importance of the two factors was to assign various values to each and select the combination which gave the best fit. The results of the tests of the super and standard control samples from mid-September till the end of December were first compared as a whole. From these grouped results the times by which 5 per cent., 10 per cent., 20 per cent., . . . of the experimental flies were dead were derived. These mortality-time relationships were then plotted and a smooth sigmoid curve, representing the survival of flies after contact with the super cloth was drawn through the points (fig. 2). Table IX explains the

TABLE IX.

Comparison of the efficiency and rate of reaction of the standard cloth with that of the super cloth.

% Tsetse Dead	Time according to mortality from			Time according to smooth curve for mortality from Super Hessian	Smooth curve which fits data from tests of Standard Hessian		Standard mortality Super mortality at $\frac{1}{2}$ time
	True Controls	Standard Hessian	Super Hessian		Time $\times 1.25$	% Tsetse Dead	
5	hrs. 5.6	hrs. 2.0	hrs. 1.5	hrs. 1.5	hrs. 1.87	4.1	82
10	6.7	2.4	1.8	1.8	2.25	8.2	82
20	9.3	2.9	2.3	2.26	2.82	16.4	82
30	12 approx.	3.3	2.6	2.6	3.25	24.6	82
40		3.8	2.9	2.84	3.55	32.8	82
50		4.2	3.0	3.05	3.81	41	82
60		4.7	3.3	3.26	4.08	49.2	82
70		5.5	3.6	3.6	4.50	57.4	82
80		6.6	4.0	4.0	5.00	65.6	82
90		8.5	4.7	4.7	5.88	75	83
95		10.0	5.3	5.3	6.63	81	85

Results of all the tests made between 17th September and 31st December on the protected control samples of DDT-impregnated hessian.

subsequent procedure. In the fifth column the times are given, slightly corrected according to the smooth curve, for the death of the specified percentages of flies from the super cloth. It is evident from fig. 2 at (a) that the efficiency of the standard cloth, as determined by the ratio of the standard mortality to the super mortality, is very much greater at four than at three hours after contact and that it increases with the increase of the survival time at which it is measured. In order to get a more constant value for this ratio the mortality from the super cloth at any given time must be measured against the mortality from the standard cloth at a later time. This ratio was found to be most constant when the ratio of the times at which the standard and super mortalities were

measured was 1.25 : 1, e.g., the ratio $\frac{65.6 \text{ per cent. at 5 hours}}{80 \text{ per cent. at 4 hours}}$ shown in the diagram at (b). The sixth and seventh columns of the Table give the data for points on a curve which is seen to be a very good fit for the mortality of the flies tested on the standard cloth, while the last column is an estimate of the percentage of efficiency of the standard cloth from the formula

$$\frac{\text{Standard mortality at time } 1.25x}{\text{Super mortality at time } (x)} \times 100$$

Corresponding to 90 and 95 per cent. mortality from the super cloth at 4.7 and 5.3 hours respectively, the mortalities from the standard cloth are 75 and 81 per cent. at 5.85 and 6.63 hours. These figures give relative efficiencies of 83 and 85 per cent. respectively, a slight rise from the constant value of 82 per cent. which holds good up to 65.6 per cent. mortality from the standard cloth, five hours after contact. After this the first signs of appreciable mortality among the control flies occurs and by 6.7 hours 10 per cent. of these are dead. This would have had no measurable effect on the rate at which the flies from the super cloth were dying.

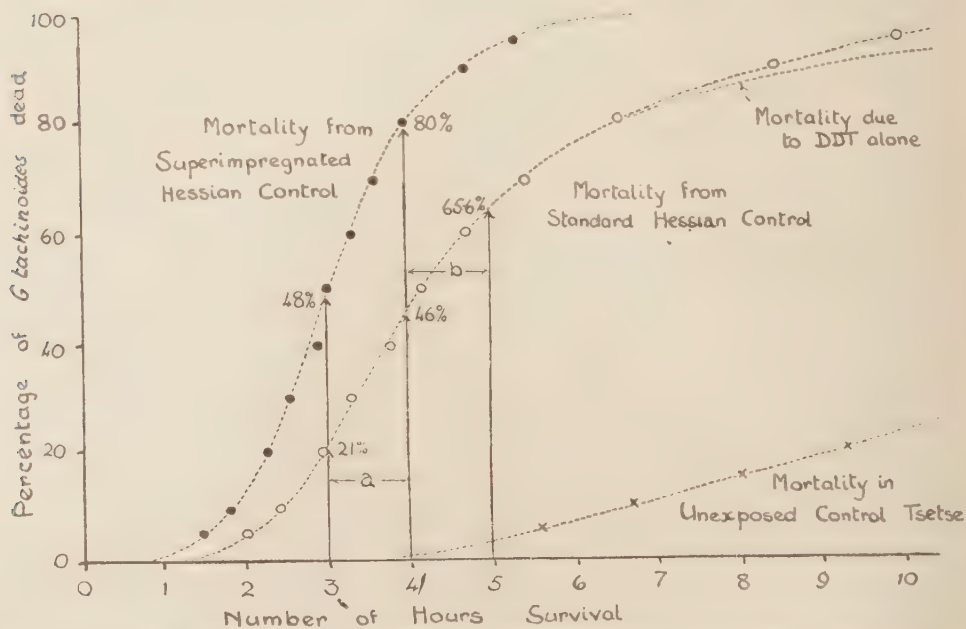


Fig. 2.—The difference between standard impregnated hessian and super-impregnated hessian according to the mortality of *G. tachinoides* after 30-second contacts. The diagram is based on the results of all the tests made between 17 September and 31 December on the protected control samples of cloth. The percentages at (a) and (b) illustrate assessments of the differences between the samples.

they were already nearly all poisoned before death from exhaustion could supervene. If, however, the mortality of flies from the standard cloth, represented by 81 per cent at 6.63 hours on the smooth curve, is corrected to allow for the mortality of the controls at this time the true mortality is 78.9 per cent. which gives an 83 per cent. efficiency. On the assumption that flies poisoned by contact with the standard cloth have on the whole a 25 per cent. longer survival than those poisoned by contact with the super cloth, then the efficiency of the standard cloth is 82 per cent. of that

of the super cloth and this relation is true throughout the whole range of the data ; it makes no difference what percentage mortality is used as the starting point for the comparison.

For this measure of efficiency to be valid it had obviously to be applicable to the monthly sections of the data as well as to the whole. Owing to the smaller numbers of results involved, the points representing the successive percentage mortalities fitted less closely to the smooth curves drawn through them, but in each case a definite relation between the curves for the mortalities from super and standard cloth was arrived at. It was found that at all times the standard cloth showed an 82 per cent. efficiency relative to the super cloth but that the survival times of the flies it killed varied relatively to those of flies killed by the super cloth, according to the season. The ratios were as follows :—

Standard Cloth : Super Cloth					
September	1.15
October	1.5
November	1.2
December...	1.25

The inference is that weather conditions, which have been termed adverse as regards effectiveness of DDT, simply cause a slower reaction on the part of the poisoned flies particularly in the case of those which have had contact with a lower concentration of the insecticide. It affords an explanation for the anomaly of impregnated cloth having apparently lost a high proportion of its toxicity within a few weeks and then somehow having regained it in a more permanent degree. Such a phenomenon might occur in the case of DDT sprayed on the porous surface of wall-board, in which the surface layer of insecticide may be brushed away and subsequently replaced by crystallisation from the deeper layers, but it seems unlikely to happen on coarse, open-textured material such as hessian.

Estimate of loss of efficiency in exposed samples of hessian.

Finally, this analytical technique can be used to give a measure of the loss of toxicity with time from pieces of impregnated hessian exposed to the weather. The lower section of Table VII gives the times of 10 per cent., 20 per cent., . . . 90 per cent., 95 per cent. mortality according to the smooth curves which have been drawn through the points derived directly from the data and shown in the upper section of the Table. Figure 3, showing the case for the super-impregnated cloth in March will serve as an illustration. The smooth curves representing the mortalities from the control sample and of the unexposed flies were drawn first. The mortality from the control sample was very far advanced by the time that deaths were beginning to occur among the unexposed flies and it was therefore not influenced to any appreciable extent by the factors affecting the condition of the unexposed flies. The curve for the mortality from the exposed sample of cloth is to some extent derived from the curve for the control sample but the first stage is to deduct from the observed mortality that which is due to environmental conditions. The mortalities of the experimental and unexposed flies are taken at various times between six hours and nine hours and, from these, the mortalities due to DDT alone are calculated in the normal way.

The new mortality curve is the one which has to be related to mortality from the control hessian. This process, although empirical, is very simple. Any particular point on the mortality curve for the control hessian can be related to a range of points on the curve for the exposed hessian. Take the relations between 60 per cent. mortality at 3.6 hours on the former and the points between (60 per cent., 5 hours) and (31 per cent., 3.6 hours) on the latter (see fig. 3). In the limit of the first case the

exposed cloth would be said to have an efficiency equal to that of the control, but the reaction would be $\frac{5}{3.6} = 1.33$ times as slow. In the second limit the efficiency would be $\frac{31}{60}$ or 51.7 per cent. of that of the control cloth within the same space of time. An intermediate point (50 per cent., 4.6 hours) on the curve for the exposed cloth would represent 83.3 per cent. efficiency in 1.09 time when related to (60 per cent., 3.6 hours) on the control cloth curve, and other intermediate points would represent greater or lesser relative efficiencies within greater or lesser time limits.

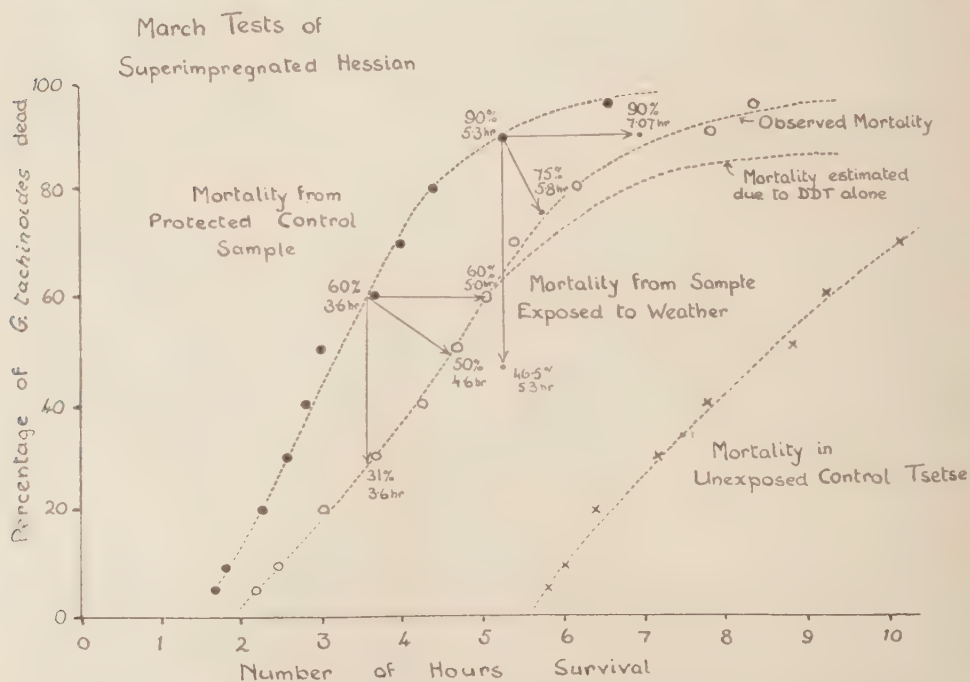


Fig. 3.—The effect of six months' exposure to weather on a sample of superimpregnated hessian according to the mortality of *G. tachinoides* after 30-second contacts with exposed and control samples of the cloth. The diagram also illustrates a method of assessing loss of toxicity described in the text.

Take another point, e.g. (90 per cent., 5.3 hours), on the control curve. With the upper limit of 100 per cent. efficiency and a time ratio of 1.33 the corresponding point for the mortality from the exposed cloth would be (90 per cent., 7.07 hours) which obviously will not meet the case. An efficiency of 51.7 per cent. within the same time limit gives 46.5 per cent. at 5.3 hours which is even worse, but 83.3 per cent. efficiency in 1.09 time gives 75 per cent. at 5.78 hours which is very close to the curve. What is really required, however, is a particular relation which, when applied to any point on the mortality curve from the control cloth, will give a point which is on or reasonably close to the mortality curve from the exposed cloth, and for this 90 per cent. efficiency with a time ratio of 1.3 was found to be quite satisfactory. Corresponding to mortalities of 10 per cent. at 1.85 hours, 20 per cent. at 2.25 hours, 30 per cent. at 2.6 hours, etc., from the control cloth, the calculated expected mortalities from the exposed cloth would be 9 per cent. at 2.4 hours, 18 per cent. at 2.93 hours, 27 per cent. at 3.38 hours, etc. In the diagram these points

fit satisfactorily a smooth curve representing actual mortality data from the exposed cloth. From this it will be seen that after six months' exposure the sample of super-impregnated hessian retained 90 per cent. of its efficiency, based on the proportion of flies it could kill with certainty, and the survival times of the poisoned flies were on the whole only 0.3 as long again as those from the protected control sample.

This method of analysis was applied to the group of data from the earlier tests with the super-impregnated and standard cloth. In two cases, October for the super cloth, and November for the standard cloth, irregularities in the data made the assessment of a definite relation of efficiency and time ratio for linking the mortality from the control cloth with that from the exposed cloth more difficult, and the choice was determined by what would best fit in with the other results which were all quite clear cut. Table X shows the loss of efficiency of the two exposed samples

TABLE X.

Loss of efficiency of samples of "super" and "standard" impregnated hessian. Mortality of *G. tachinoides* after 30-second contacts compared with mortality from protected control samples.

Tests carried out between	Exposure of impregnated cloth to weather	Super		Standard		Exposed standard referred to super control	
		Efficiency of exposed cloth relative to control sample		Efficiency of exposed cloth relative to control sample		Relative killing power	Relative time for tsetse to react
		Relative killing power	Relative time for tsetse to react	Relative killing power	Relative time for tsetse to react		
	weeks	%	times as slow	%	times as slow	%	times as slow
Sept. 17 and 30...	0-2	100	1.5	98	1.6	81	1.84
Oct. 6 and 16...	3-4	97	1.7	95	1.8	78	2.7
Oct. 28 and Nov. 6...	6-7	95	1.25	92	1.25	75	1.5
Dec. 1 and 30...	11-15	93	1.1	90	1.1	74	1.2
Mar. 1 and 14...	24-26	90	1.3				

of cloth. Each is expressed in terms of its own control and in addition the standard cloth is given in terms of the super control. It can be seen that according to the estimate the loss of efficiency is steadily progressive and, in so far as this is so, the method of comparing the test samples of impregnated hessian with the controls is satisfactory and it is more exact than the simpler conventional methods.

The factors which in the other methods of comparison suggested irregular changes in efficiency are now restricted to the relative proportion of flies killed and the relative time taken by the flies to react to the effects of DDT poisoning. The first factor, percentage efficiency, is dependent on the state of the cloth and this is something that, logically, would be expected to deteriorate with the passing of time. The second depends mainly on the state of the flies and represents the combined influence of the physical conditions of the flies themselves (whether they are on the whole larger or smaller) and the atmospheric conditions of their environment, the experimental environment in particular. This is extremely important in any practical application of the test. If the experiments had been carried out under ideal laboratory

conditions, it might have been possible to obtain uniform, artificially bred, stocks of flies maintained in unvarying conditions of temperature and humidity and to have exposed them to samples of cloth under these same conditions. This would have simplified the estimation of the persistence of toxicity in the impregnated hessian but it would have given no clue to the reaction of tsetse to such material in the field.

The Use of DDT-impregnated Hessian on Tsetse Traps.

Once it was known that certain applications of DDT to hessian were definitely more toxic to tsetse after very brief contacts, and that the toxicity persisted in measurable degree over as much as six months, it was possible to design experiments incorporating DDT with a view to increasing the efficiency of tsetse traps. The object of adding an insecticide to a trap is to exterminate those tsetse which have been attracted to the trap and have walked about on it but have been either too wary to penetrate the interior or able to escape from the interior. There was, however, the possibility that the mere presence of the DDT would defeat this aim. Kennedy (1947) showed in experiments with *Anopheles maculipennis* Mg. and *Aedes aegypti* (L.) that contact with DDT-treated surfaces excites mosquitos and renders them liable to fly off and escape without having absorbed a lethal dose. In Lagos, Muirhead Thompson (1947) proved that this irritant effect of DDT-treated surfaces is an important factor and suggested that it may greatly reduce the value of the treatment of houses with DDT as a control measure against *Anopheles gambiae* Giles. Although the general behaviour of the tsetse shows less irritability and more deliberation than the mosquito, the tsetse were often restless during their experimental contact with DDT and they would rub their tarsi together as if trying to clean them. And, although they could readily be made to spend the required interval on the hessian when imprisoned in the test-tubes, there was no guarantee that they would do so if perfectly free to do otherwise. There was also the possibility that the strong smell of kerosene in freshly prepared hessian, which is faintly perceptible for quite a time afterwards, would repel the tsetse and destroy the illusion of the trap as a host animal.

Four traps of the standard animal pattern were placed in positions of good visibility in a tsetse feeding ground on the Black Volta River. Two of the traps were covered in the normal way with natural hessian and the third with black hessian. The natural hessian covering of the fourth had been soaked in a kerosene solution of DDT and was equivalent to the standard cloth of the toxicity tests. Three times a week the traps were inspected and their catches recorded. The traps were interchanged weekly in order to eliminate the factor of variability of sites from the results, in such a way that by the end of a four-week period each trap had occupied each site for a week.

The traps were in operation from 31st July till 18th December, 1948, the total numbers of tsetse caught being :—

		<i>G. palpalis</i>	<i>G. tachinoides</i>
Natural Hessian Trap (1)	...	51	345
" " " (2)	...	71	379
Black " " "	...	47	343
Natural " with DDT	...	76	514

So, far from the presence of the DDT having acted as a repellent or otherwise reduced efficiency, the impregnated trap was superior to the other three in respect of both *G. palpalis* and *G. tachinoides* and its total catch was 43 per cent. greater than the average of the others. To judge from the uniformity among the control trap catches

it is unlikely that this particular trap was intrinsically more efficient than the others. The numbers of flies caught in the successive four-weekly periods were as follows :—

				Trap with DDT	Average of Control Traps	% Additional efficiency of Trap with DDT
31 July	—28 August	171	83	106
28 August	—25 September	68	61	11
25 September	—23 October	25	34	—26
23 October	—20 November	137	102	34
20 November	—18 December	189	130	45

Since the difference is most marked in the first period when the DDT was fresh, it is safe to assume that the insecticide played some positive part in the result recorded. There is no evidence that DDT has any attraction for tsetse so if it did in fact help to catch them the explanation must be that the onset of toxic symptoms was sufficiently delayed to have no effect on the flies' continued exploration and entry into the body of the trap, nor did they interfere with the flies' tendency to move up towards the light and so into the cage; inside the cage the flies buzz restlessly trying to escape and here the onset of paralysis takes its toll. The trap-boy reported that while he quite often found flies still alive in the cages of the ordinary traps when he made his records, those in the DDT trap were nearly always dead. The implication is that in the case of an ordinary trap an unsuspectedly high proportion of the flies manages to escape again, probably in the early morning or the evening when they are no longer phototropically drawn to the top of the cage. To this effect which is measurable must be added the unknown mortality amongst those flies which have explored the trap without entering the catching cage. Provided that the trap is adequately impregnated the great majority of these will have received a lethal dose of DDT. Thus an animal trap which has been impregnated with DDT is definitely superior to one which has not been so treated.

The seasonal modification in the relative efficiency of the impregnated trap corresponds satisfactorily with the results of the laboratory tests. The loss in September of most of the initial superiority of traps, followed by a negative result in October, occurred at the same time that the experimental flies from the test samples of both super and standard cloth and from the standard control cloth were showing temporarily increased survival times. In the case of the traps, the DDT-treated hessian was 2–3 months old and in the toxicity experiments it was only 3–5 weeks old, consequently the effect cannot be ascribed to an ageing of the DDT films. In the tests for the effect of moisture on impregnated hessian a similar slowed reaction was observed in the case of cloth from which the moisture had almost entirely evaporated. This confirms the view that there are certain climatic conditions under which DDT preparations become less effective against tsetse. On the other hand, the increased susceptibility of the flies to the insecticide under hotter and drier conditions is well shown. The trap covers were well saturated with dust by the end of the test but, despite this, the DDT was still proving effective.

During the writer's absence on leave, animal traps impregnated with a 25 per cent. emulsion of DDT (ATSO) were in use at crossing places on the Black Volta. Unfortunately there were no untreated controls but the data from the traps exposed is of some interest because it gives an indication of the relative merits of standard cloth as a cover and hessian sprayed with DDT emulsion. In each case two traps were alternated on two sites week by week and their catches recorded twice weekly.

For the 18-week period 3rd January-7th May, 1948, the total catches at one place were :—

	<i>G. palpalis</i>	<i>G. tachinoides</i>
Trap 1, Trap covered with "Standard" DDT hessian ...	14	831
Trap 2, Hessian cover sprayed with DDT emulsion ...	14	1,166

It will be seen from the above figures that hessian sprayed with emulsion showed a superiority of 46 per cent. over the standard hessian. The emulsion trap caught twice as many flies as the latter in the first month; later its superiority was less but not in any definitely diminishing relationship. At another place, the catches between 8th January and 10th May gave altogether a 36 per cent. superiority for emulsion over kerosene solution but in this case a diminishing trend was evident.

	Trap 3 covered with "Standard" DDT hessian	Trap 4 cover sprayed with DDT emulsion	Emulsion catches as percentage Standard DDT catches	Total Rainfall ins.
	Total catches			
8 Jan. - 5 Feb. ...	641	1,149	179	—
5 Feb. - 4 Mar. ...	634	871	137	—
4 Mar. - 1 Apr. ...	474	509	107	—
1 Apr. -29 Apr. ...	297	308	103	2.33
29 Apr. -10 May ...	106	94	89	4.70

Traps 1 and 3 were washed away by a flood and Trap 4, its cover resprayed with emulsion, was transferred to work in conjunction with Trap 2. The catches for the ten-week period 21st May-7th July were :—

	<i>G. palpalis</i>	<i>G. tachinoides</i>
Trap 2 sprayed DDT emulsion 2 Jan. ...	41	709
Trap 4 sprayed DDT emulsion 2 Jan., resprayed 19 May ...	53	1,143

This superiority of 59 per cent. for Trap 4 suggests, either that the DDT prepared at the beginning of the year had become greatly reduced in its effect, or that the second impregnation of Trap 4 was a heavy one. It is interesting to see the progressive reduction in the relative value of the Trap 2 catches.

	Trap 2 sprayed DDT emulsion 2 January	Trap 4 sprayed DDT emulsion 2 January, resprayed 19 May	Trap 2 catches as percentage of Trap 4 catches	Total Rainfall ins.
21 May - 4 June ...	133	177	76	1.01
4 June -18 June ...	213	302	71	6.97
18 June - 2 July ...	149	247	61	7.78
2 July -16 July ...	160	273	58	9.60
16 July -30 July ...	95	197	48	10.74

At the beginning of August both traps were re-covered with freshly impregnated hessian. The catches for the 16 weeks 13th August–3rd December were :—

	<i>G. palpalis</i>	<i>G. tachinoides</i>
Trap 2.—Trap covered with Standard DDT hessian ...	92	1,584
Trap 4.—Hessian cover sprayed with DDT emulsion	39	1,108

In this case, the catches in the emulsion trap were altogether 31 per cent. less than those from the standard DDT trap. The application of the emulsion by spraying probably resulted in a diversity in the total amount of insecticide used and this may partly have accounted for the difference between this and the earlier results. But exposure to rain undoubtedly had some effect also.

	Trap 2 covered with Standard DDT hessian	Trap 4 cover sprayed with DDT emulsion	Emulsion catches as percentage of Standard DDT catches	Total Rainfall
	Total catches			ins.
13 Aug. –27 Aug. ...	154	170	110	2.03
27 Aug. –10 Sept. ...	122	114	93	8.76
10 Sept. –24 Sept. ...	133	111	83	12.09
24 Sept. – 8 Oct. ...	323	215	67	12.79
8 Oct. –22 Oct. ...	321	184	57	15.08
22 Oct. – 5 Nov. ...	202	125	62	15.47
5 Nov. –19 Nov. ...	152	85	56	15.47
19 Nov. – 3 Dec. ...	269	143	53	15.47

The emulsion trap started with a slight initial advantage but lost this and soon became inferior to the standard DDT trap. It can be seen that the rate of decline fell off as the rainy season drew to a close. How much of its own additional efficiency the standard DDT trap lost in that period is of course not known in the absence of records from a control trap, but it is certain from the laboratory tests that it must have retained a useful proportion of its toxicity to the end. Emulsion-treated cloth had previously been tested in the dry season only and it is possible that it does not retain its toxicity under wet season conditions. Additional field tests with this and similar preparations of DDT are in progress.

Conclusions.

The most striking features of the laboratory tests were the great variation among survival times of tsetse after contact with DDT and the fact that these survivals lengthened considerably in October and then diminished until in December they were shorter than they had been originally. This is considered to be due to the effects of changing weather, directly in the sense that with certain meteorological conditions either the reaction of the flies to DDT slows down or the action of the DDT is at least partially inhibited ; indirectly because with the continuation of the dry season, the flies were smaller and so likely to be more vulnerable. In the experiments with traps, the increase in catches after the application of DDT must be due to the onset of toxic symptoms interfering with the flies' attempts to escape, thus reducing what must normally be a substantial leakage. Thus a variation in the time taken for the immobilisation of the flies will bring a corresponding variation

in the superiority of impregnated traps. It is highly significant that there was seasonal agreement in this respect between laboratory and field experiments.

An emulsifiable preparation of DDT has also been shown to increase the efficiency of traps and to retain a high degree of toxicity for at least three months in dry season conditions. The use of an emulsion rather than a kerosene solution would add greatly to the convenience and safety of impregnating hessian for trap covers in an extensive employment of this means of protection and it would also reduce the cost of the operation.

The experiments indicate the possibility of using traps as a means of testing delayed-action insecticides. The outstanding advantage of such a method is that the tests are being made under natural conditions in the tsetse's habitat. Initial laboratory tests are necessary to show the action of the insecticide on the flies, but the effect and durability of various strengths can be assessed from regular and prolonged trapping records. By this means a number of tests can be carried out simultaneously in the field with routine supervision by one intelligent trap-boy. Investigations of this kind are now in progress in Lawra. Essential precautions to ensure uniformity and reliability of the traps involve 3-4 weeks' test before the application of the insecticide and systematic interchanging of the traps on the sites.

The attractant qualities of the animal trap to tsetse provide a good basis for the efficient use of DDT in their control. If the insecticide is applied by soaking the hessian thoroughly in a kerosene solution, it will withstand a considerable amount of rain, and a really heavy deposit will remain lethal to tsetse for four to six months without the necessity for re-treatment. To use DDT liberally in particular and specialised situations known to attract the tsetse is very much more satisfactory than to attempt to kill off the last fly by broadcasting the insecticide in the hope of rendering all the habitats lethal. Practical considerations support this concept. During the dry season, both people and tsetse concentrate in the vicinity of available sources of water and the tsetse, suffering from water shortage, have to feed more frequently: if cases of trypanosomiasis are present, the risk of transmission would appear to be at its greatest. Traps have been proved to catch most successfully in the dry season, and DDT has been shown to have the strongest effect on the tsetse at this same time. Wherever tsetse traps serve a useful purpose, not only can DDT, if properly applied, improve the traps, but it will kill tsetse without the risk of upsetting the balance of nature by indiscriminate slaughter among the whole insect population.

Summary.

Experiments were carried out to investigate the persistence of toxicity to tsetse of hessian, impregnated with DDT, exposed to the normal weather conditions of the Inland Savanna Region of the Gold Coast; also, to establish a satisfactory method of estimating the loss or change in its toxicity, and to study the effects of impregnation with DDT on the efficiency of tsetse traps.

Hessian was impregnated by being soaked for ten minutes in a filtered saturated solution of commercial DDT in kerosene which gave 4.4 g. DDT per sq. ft. and tests were made at intervals with batches of *Glossina tachinoides* at 15- and 60-second contacts. The comparison of the survival times of these flies compared with those of controls showed an appreciable residual toxicity after 20 weeks' full exposure to the weather including 4.26 ins. of rain, a slight toxicity after 30 weeks, and a negligible toxicity after one year including 34.35 ins. of rain. The same preparation could still produce toxic symptoms in tsetse after 15-second contacts when exposed for one year to wind and daylight, but protected from rain and direct sunlight. There was no great difference on the whole between the results from 15-second and 60-second contacts.

It was found that survival times lengthened in October when the humidity was high but were shortened in December when the conditions became hotter and drier.

A further experiment was carried out with hessian carrying 4.4 g. DDT per sq. ft. and super-impregnated hessian with 7.4 g. DDT per sq. ft. Samples of both were exposed to the weather and others kept as controls. All samples were tested by 30-second contacts with *G. tachinoides*.

It was shown that contact with the super-treated cloth was invariably fatal; the standard cloth had an 82 per cent. toxicity but the flies that received a lethal dose took longer to die on the whole. It was found to be the survival time and not the toxicity that varied with seasonal conditions.

A full analysis of the survival times from the exposed samples in relation to those from the control samples showed that exposure to weather during late rains followed by dry season conditions caused only a slight but progressive deterioration of the killing power of exposed DDT-impregnated hessian. This amounted to 10 per cent. in 3-4 months including 7.42 ins. of rain in the case of standard cloth, and 10 per cent. in six months with the same amount of rain in the case of the super cloth.

Hessian impregnated with 25 per cent., 50 per cent. and neat DDT emulsion was found to be highly toxic and to retain its efficiency satisfactorily for at least 12 weeks' exposure in the dry season.

Hessian freshly impregnated with DDT in kerosene solution caused flies to die more quickly as long as traces of kerosene persisted but moisture, on the other hand, interfered with the action of DDT unless kerosene was also present.

An "animal" tsetse trap covered with standard impregnated hessian showed a 40 per cent. superiority in the numbers of *G. palpalis* and *G. tachinoides* taken over a period of 20 weeks. Corresponding to the results of the laboratory tests this superiority was lost in October but reappeared in December.

Comparisons of catches made by traps covered with standard impregnated hessian and hessian sprayed with 25 per cent. ATSO emulsion showed that, although the emulsion might initially give much better results, its superiority relative to the standard impregnation tended to diminish, especially during the wet season.

The increased efficiency of an impregnated trap is more than the measurable amount of the increased catches since flies which have investigated the trap, but have not been caught, are likely to have received a lethal dose of DDT.

The application of DDT to traps represents an economical use of the insecticide which can conveniently be applied in sufficient concentration to ensure that brief contacts will be fatal to tsetse over a period of several months.

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References.

- GAHAN, J. B., TRAVIS, B. V. & LINDQUIST, A. W. (1945). DDT as a residual-type spray to control disease-carrying mosquitos; laboratory tests.—J. econ. Ent., **38**, pp. 236–240.
- KENNEDY, J. S. (1947). The excitant and repellent effects on mosquitos of sub-lethal contacts with DDT.—Bull. ent. Res., **37**, pp. 593–607.
- MORRIS, K. R.S. (1946). The control of trypanosomiasis by entomological means.—Bull. ent. Res., **37**, pp. 201–250.
- MORRIS, K. R. S. & MORRIS, M. G. (1949). The use of traps against tsetse in West Africa.—Bull. ent. Res., **39**, pp. 491–528.
- MUIRHEAD THOMSON, R. C. (1947). The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa.—Bull. ent. Res., **38**, pp. 449–464.
- PARKIN, E. A. & GREEN, A. A. (1947). DDT residual films. 1. The persistence and toxicity of deposits from kerosene solutions on wall-board.—Bull. ent. Res., **38**, pp. 311–325.
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SIMPLE TESTS ON THE EFFECTIVENESS OF SOME CHLORINATED HYDROCARBON INSECTICIDES AGAINST THE LEATHER BEETLE (*DERMESTES MACULATUS* DEG.).

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Numerous methods for testing insecticides have been developed in recent years (Gnadinger, 1936, 1945 and Webb, 1947), all capable of giving highly accurate results, but all requiring apparatus and skill only to be found in research institutes with the necessary materials and specialist staff. Such institutes are, for the most part, not interested in the industrial aspects of pest control.

In order that the leather and similar industries may have some means of learning whether a new insecticide is suited to their own particular needs, it is desirable to develop a simple and rapid test, applicable to the conditions of the industrial laboratory, which will be more reliable than *ad hoc* field trials. The object of the present work was to investigate the possibility of designing such a test while at the same time comparing the potency of some of the newer contact insecticides towards the primary pest of the leather industry, *Dermestes maculatus* Deg.

Three chlorinated hydrocarbon insecticides were selected for comparison, two of which have already been the subject of field trials by Kritzinger (1946, 1947) and Bovingdon & Stock (1947). It became clear from preliminary experiments that a test could be only elaborated if the production of an evenly spread film, varying by not more than 0.2 μg . per sq. cm. of surface, could be guaranteed; the more potent insecticides caused high variations in mortality at film densities with variations greater than this, and one of the insecticides caused immobilisation of the test insects so that the influence of film density was restricted to a very small area. In the present work, variations in average spread have been within 10 per cent. of the calculated value where the deposit was thick enough to be weighed, and the indications are that factors other than film density are causing the main divergences in the results.

Insects and Insecticides.

Insects were bred in wide-mouthed jars or tins containing sterilised fish meal and dried yeast in the proportions of 5-7 parts of meal to 1 of yeast. Water was supplied by small pieces of damp cotton wool which were renewed at intervals at the discretion of the observer. Cultures were kept in an incubator at $27 \pm 1^\circ\text{C}$. Insects required for tests were removed at random from any one or several of the jars. At one stage it became apparent that conditions in certain of the cultures were below the optimum and were influencing the quality of the test insects. In the absence of a large-scale breeding chamber, precautions were taken later to minimise differences due to breeding and, when possible, to select insects from several different jars for each test.

Pure samples of the following insecticides were compared:—

- (1) *p,p'*-dichlorodiphenyltrichloroethane (DDT) m.p. 109°C . phenyl
- (2) γ -isomer of 1,2,3,4,5,6-hexachlorocyclohexane (Gammexane sample), m.p. 112.5°C .
- (3) 2,3,4,5,6,7,8,8, - octachloro - 4,7 - methano - 3a,4,7,7a, - tetrahydroindane (Chlordane) a dark-brown, highly viscous liquid. 5/

Spread and Variability of Films.

In preliminary experiments, films were formed by applying drops of solutions of benzene hexachloride or DDT in volatile organic solvents to the centres of filter papers and allowing the solutions to spread and evaporate. Concentric sections were cut from the filter papers treated with DDT to test the evenness of distribution and the DDT determined by the method of Bradbury, Higgins, and Stoneman (1947). The results showed that the concentration decreased regularly from the centre to the edge of each paper; additions of a DDT solution calculated to give 0.02 mg. per sq. cm. produced concentrations of 0.04 mg. per sq. cm. in the innermost circle of 1 cm. radius, down to 0.0007 mg. per sq. cm. at the edges. Moreover, the precise amount of insecticide available to the insects was uncertain since it was deposited throughout the pores of the paper on both surfaces. Busvine and Barnes (1947) used filter papers in tests with benzene hexachloride and DDT and attempted to overcome unevenness of spread by applying the solutions at several points but concluded that the surfaces produced leave much to be desired. For these reasons the filter paper was abandoned as a test medium and the remaining trials were carried out on glass photographic quarter-plates, and tests with Chlordane were included.

A number of solvents were tested for their ability to form an even film on glass. Those, such as kerosene, with a high boiling point, evaporated very slowly and were less convenient for regular use than those boiling from 60–100°C. Chloroform was finally selected as giving a reasonably good film, provided the glass plates were dry. Nevertheless, good films were difficult to prepare and the most satisfactory procedure was to pipette a known volume of solution from a graduated 1 ml. syringe and to spread it across the plate by means of a microscope slide as in the preparation of blood smears. The amount of solution retained by the microscope slide was approximately 5 per cent. of the total and, except for a slight thickening at the end of the smear, the resulting film appeared evenly spread and, although it was not always continuous, each half had a similar toxicity. The thick part was shown by weighings before and after its removal with chloroform to contain about 10 per cent. more insecticide than the remainder of the film, so that a total error of some 15 per cent. could be expected in the very thin films of benzene hexachloride and Chlordane. In the DDT films, which were much denser and heavier, such errors were minimised by removing the thick edges apparent to the naked eye, and by determining the film concentration by weight difference.

A further unavoidable error in the tests arose from the volatility of two of the insecticides used. It was established that, in six hours, films of benzene hexachloride and Chlordane exposed to the still air of the test cabinet lost 0.012 and 0.0018 mg. per sq. cm., respectively, so that the effective doses were actually less than those calculated. A compensating, although further complicating, factor was the toxicity of the vapour of benzene hexachloride to *Dermestes*. Exposure to vapour-saturated air at 25°C. for six hours killed 56 insects out of 100 and a film of benzene hexachloride covered with a double layer of muslin killed eight out of 12 insects retained on the muslin for the same time whereas no insects were killed after a similar test with a highly concentrated film of DDT. Chlordane vapour possessed little toxicity and merely immobilised the insects exposed to it.

As would be expected, benzene hexachloride and Chlordane films lose their effectiveness with increasing age, although mortality figures obtained for benzene hexachloride after one day's ageing did not differ significantly from those with fresh films at the higher concentrations used. No increased toxicity was noted with old DDT films such as has been reported by Parkin and Green (1945) who also demonstrated that film toxicity depends on the method of deposition. They found that films equal in surface density, prepared from solutions of different concentrations, differed in toxicity; the toxicity of films prepared by a single deposition also differed from that of films deposited in several stages. In the present series of tests, the limitations

of the spreading technique prevented solutions of the same concentrations always being used, since the volume of liquid spread could not easily exceed 0.2 ml.

Dosage mortality relationships.

In the first series of experiments, adult insects were confined in a cabinet at $25 \pm 3^\circ\text{C}$. by means of inverted glass funnels for six or 16 hours on the films of insecticide spread on plates. The highest possible concentration of DDT had no appreciable effect in six hours, and the smallest effective concentrations of benzene hexachloride and Chlordane volatilised long before the completion of the test in 16 hours. It was, therefore, not possible to use the same period of exposure for the comparison of these three insecticides. Further experiments gave widely divergent results, and efforts were made to improve replication by maintaining the temperature more nearly constant and by leaving the insects in clean dishes for a sufficient period after treatment to enable the complete effects to become apparent. In addition to the number of insects killed, those seriously affected were noted, but after 120 hours these became sufficiently rare to be neglected. It was not judged necessary to adopt any criterion of death other than that of simple observation, since experience showed that insects affected so severely as to lose all power of movement did not recover. As data from small-scale trials accumulated and still gave variable results, efforts were made to improve the test by studying the influence of sex, size, and age of the insects as well as the effect of the age and crystal size of the insecticide films. The results of the standardisation tests are described below. The data obtained from the plate tests, on which the estimates of relative toxicity are based, are shown in Table I, and plotted graphically in fig. 1. Most of the trials were carried out on ten to 30 insects at each concentration, subdivided into groups of five to ten exposed to different areas of film to compensate for any differences due to slight unevenness.

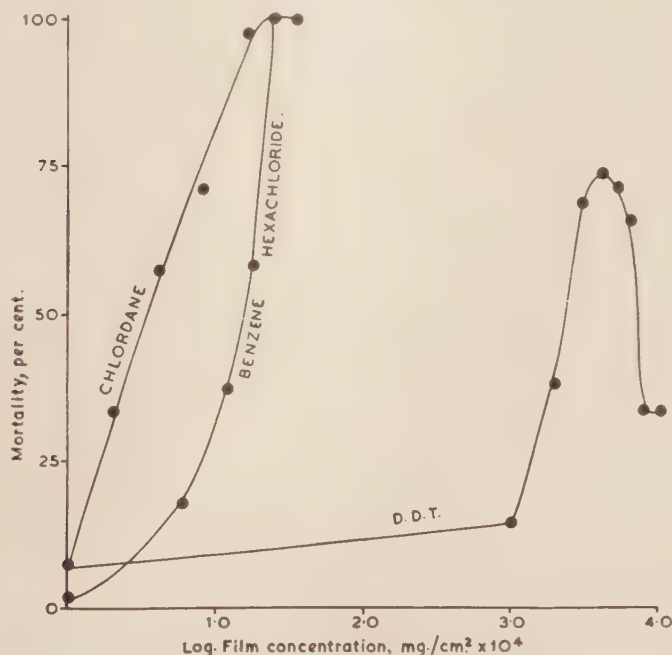


Fig. 1.—Relationship between mortality and film concentrations of Chlordane, benzene hexachloride, and DDT.

TABLE I.

Relationship between insect mortality and insecticide film concentration.

Benzene hexachloride mg./sq. cm. ...	Control	0.0006	0.0012	0.0018	0.0024				
No. of insects killed ...	2/81	18/100	42/112	59/100	54/54				
Mortality, % ...	2	18	37	59	100				
DDT, mg./sq. cm.		0.1	0.2	0.3	0.4	0.5	0.6	0.7	1.0
No. of insects killed ...	15/230	10/72	76/198	131/193	67/91	120/172	97/148	71/210	28/80
Mortality, % ...	7	14	38	68	74	70	66	34	35
Chlordane, mg./sq. cm. ...		0.0002	0.0004	0.0008	0.0017	0.0033	0.0066		
No. of insects killed ...	4/60	40/101	57/101	71/101	79/81	81/81	30/30		
Mortality, % ...	7	40	57	71	97	100	100		

Preliminary tests, which gave a guide to the approximate concentration of the median lethal doses, permitted subsequent tests to be made within a fairly narrow range of concentrations. Values obtained for films prepared at the higher concentrations of DDT showed a definite decline in effectiveness, and this is almost certainly attributable to the nature of the film itself, since above concentrations of 0.4 mg. per sq. cm. a tightly packed crystal mass covers the plates and the evenness begins to be destroyed by the formation of a superimposed second layer. To investigate this apparent anomaly in toxicity, batches of 30 insects, all of equal weight to within 2 per cent., were confined on plates, selected for evenness of spread, in a constant temperature room for the standard period of 16 hours after which they were transferred to clean dishes and left for a further six days.

TABLE II.

Relationship between mortality, time after exposure, and concentration of DDT on plates.

Time after Exposure hrs.	0.21		0.32		0.40		0.49		0.60		Control	
	Killed	Total	Killed	Total	Killed	Total	Killed	Total	Killed	Total	Killed	Total
0	0	30	0	30	0	30	1	30	0	30	0	30
8	3	30	0	30	0	30	2	30	2	30	0	30
32	11	30	20	30	3	30	11	30	22	30	0	30
56	22	30	25	30	6	30	19	30	30	30	0	30
80	26	30	30	30	8	30	23	30	—	—	2	30
144	27	30	—	—	9	30	25	30	—	—	2	30
Percentage Mortality	97		10		30		83		100		7	

Results are given in Table II. From this experiment, the earlier behaviour was fully confirmed but the maximum mortality was reached at a somewhat lower concentration. It seemed possible that the addition of an upper layer of crystals was

able to eliminate or minimise the effect of the lower layer, either by a simple screening effect or by influencing the size or nature of the crystal clusters. Tests were accordingly made on films containing up to 1.2 mg. of DDT per sq. cm., all prepared from different volumes of the same concentration of DDT in chloroform, so as to avoid any differences due to concentrations such as were noted by Parkin and Green (1945). The results, after 16 hours' exposure of the insects, are shown in fig. 2. The plates again showed clearly the formation of a double film but microscopical examinations of the films themselves revealed pronounced differences in crystal structure, the films of low concentration being composed of large radiating needle clusters, and the films of higher concentrations possessing more tightly packed clusters of smaller crystals.

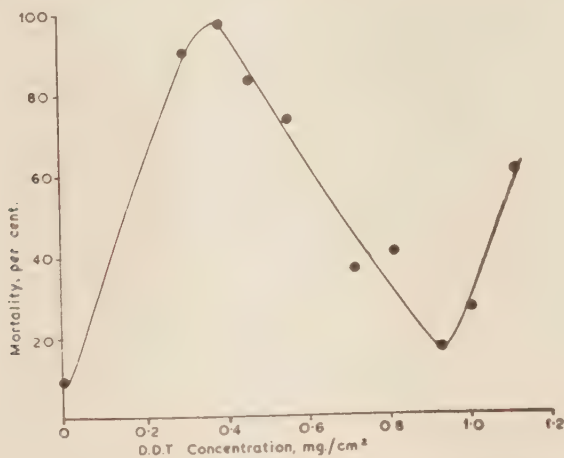


Fig. 2.—Relationship between mortality and film concentration of DDT.

Tests of Larvae.

A few small-scale tests were made with final-instar larvae of *Dermestes*, although the numbers were insufficient for accurate comparisons. The resistance of the larvae towards all three insecticides proved much greater than that of the adults, but the general picture was the same: high concentrations of DDT produced a very slight effect, whereas benzene hexachloride and Chlordane evoked a severe and lethal reaction usually at comparatively low doses (see Table III).

TABLE III.

Effect of insecticide film concentration (mg./cm.²) on mortality of *Dermestes* larvae.

Exposure time, hrs.	Benzene hexachloride		DDT						Chlordane		Control
	0.01	0.04	0.1	0.2	0.3	0.4	0.5	0.6	0.01	0.04	
20	20/20	29/29	3/6	1/6	1/6	0/6			20/20	20/20	0/6
52				15/18	6/18	12/18	7/18	8/18			3/18
62											

Tests on Eggs.

Less than one hundred eggs were used for the tests but the results were clearly defined and conclusive. After five days, eggs exposed to benzene hexachloride vapour at 25°C. became brown in the normal way as if ready to hatch, but the larvae inside were dead. Under favourable conditions eggs hatch after three days at 27°C. (Hinton, 1945). Eggs placed on a plate with a DDT film of fairly high concentration (0.22 mg. per sq. cm.) all hatched after five days at room temperature, but the larvae were killed on emergence.

Variability of insects.

The previous relationships of mortality and dosage having been obtained without regard to variations in the insect material, an investigation into the effect of these variations on mortality was thought to be a useful check on the accuracy of the tests. Accordingly, numbers of insects of known age were weighed and sexed after exposure to films of DDT in the usual way. Other insects similarly weighed and sexed were exposed to air saturated with benzene hexachloride vapour at 25°C. to increase the precision by avoiding differences in film concentration on the plates, but it was realised that exposure of the whole body surface to poisoning would not necessarily provide evidence of value in assessing the factors influencing reaction to film deposits where the locus of action is highly specific and unknown.

The weight distribution of insects used for the tests on variability was as follows :

Weight range of each insect, mg.

0-5 6-10 11-15 16-20 21-25 26-30 31-35 36-40 41-45 46-50

No. of insects.

3 40 82 83 75 31 19 4 3 1

It will be seen from the foregoing figures that, out of a total of 341 insects, almost half weighed between 11 and 20 mg., and three-quarters weighed between 6 and 25 mg. As will be seen from fig. 3, this is of importance as an indication that in the earlier trials the differences in toxicity were in direct proportion to size of insect and therefore likely to give reasonable averages for comparison.

The relationship between weight and sex is summarised in Table IV, from which it will be seen that the ratio of females to males among the smaller and more numerous insects (the sex ratio) is 1 to 5.2; above 26 mg. weight the sex ratio is 1 to 1.6, the females of *Dermestes* tending to be larger and heavier than the males.

TABLE IV.
Sex ratios in *Dermestes* of different weight groups.

Weight, mg.	Males	Females	Ratio
6-10	33	8	4.1
11-15	68	12	5.7
16-20	47	12	3.9
21-25	47	9	5.2
26-30	16	9	1.8
31-35	1	2	0.5
36-40	2	1	2.0
Total	214	53	4.0

Insects exposed to benzene hexachloride vapour and DDT films showed marked differences in susceptibility according to weight, as might be expected (figs. 3 and 4). These differences were the same whether the criterion of susceptibility was death or

the onset of the first certain toxic symptoms when the leg movements became uncoordinated, causing the insects to fall over repeatedly until they were finally unable to rise again. From the close similarity of the curves for dosage-mortality and dosage-uncoordination (fig. 3), it might be postulated that penetration through the cuticle was immediate and that the time lag before an effect was visible was due solely to the rate of reaction of the toxic agent with the internal tissues. From Table V it can be seen that only slight and insignificant differences arise

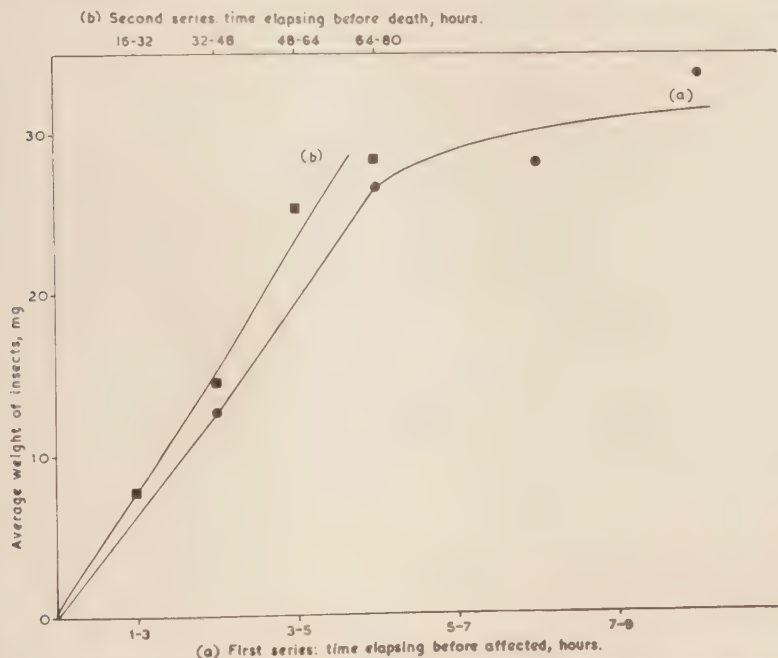


Fig. 3.—Relationship between weight of insects and reaction to benzene hexachloride vapour.

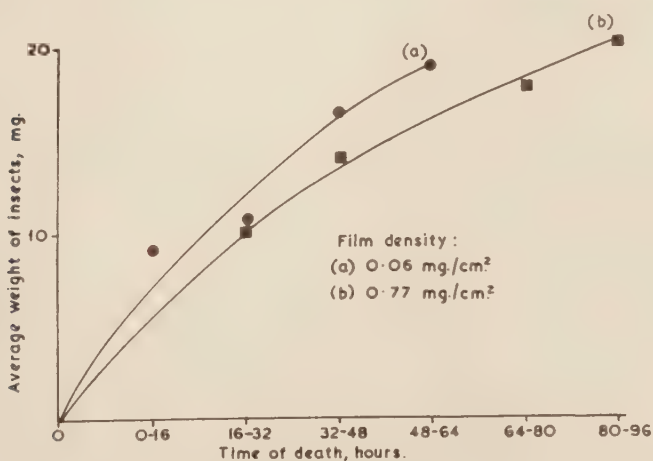


Fig. 4.—Relationship between weight of insects and mortality on DDT films.

TABLE V.
Relation between sex and susceptibility to insecticide.

		Benzene hexachloride			DDT			Proportion of males/females in normal population
		Males	Females	Ratio	Males	Females	Ratio	
Easily killed...	...	38	7	5.4	78	16	4.9	5.2
Less easily killed	...	31	12	2.6	67	24	2.8	1.6

from sex, apart from those relating to weight differences. It must be noted, however, that in comparing the sex ratios of insects of differing grades of susceptibility (Table V) with those of different weight groups (Table IV) the assumption is made that susceptibility is invariably related to weight (that is, all insects of less than 26 mg. weight would belong to the class of "easily killed", and all insects of 26 mg. or over would belong to the class of "less easily killed", whereas this is true only on average. Nevertheless, if female insects were more susceptible to insecticides than males, the proportion of females in the "less easily killed" group would be higher than the normal population for this group, and the ratio of males to females would accordingly be lower than the normal heavy weight group ratio of 1.6. Conversely, if males were the more susceptible, the difference would be evident from the proportions in the "more easily killed" group, which would then have a higher ratio than that of the normal light weight group.

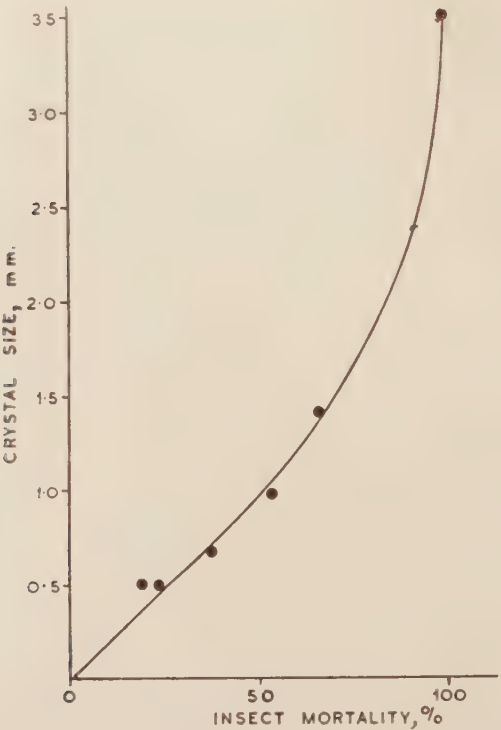


Fig. 5.—Effect of solvent on toxicity of DDT films.

Differences due to age were investigated by exposing together adult beetles of known dates of emergence to vapour or films after marking the different age groups with paint spots. It seems most unlikely, from the even distribution of the distinguishable age groups throughout the whole of the batches tested, that differences due to age can arise for the first ten days of adult life.

Despite the evidence that weight of insect is the prime cause of variation in susceptibility, an attempt was made to assess the replicability of the test by exposure of 12 batches of 5 insects of random weight to benzene hexachloride films for 30 min. The numbers killed were 5, 4, 3, 3, 1, 2, 3, 3, 4, 3, 2, 5.

The results show that large mortality differences could occur, although in comparing the first six tests with the second, the differences are not significant ($P > 0.1$).

It has already been shown, by McIntosh (1946) and Parkin & Green (1945) that crystal size plays a great part in controlling the toxicity of DDT films; this may explain the differences shown above and throughout the tests with DDT.

Variability of Film due to Solvent.

A further series of experiments was performed on DDT films prepared from 0.1 ml. quantities of a number of different 10 per cent. solutions to illustrate the relative effects of crystal differences in films of equal density. Insects were exposed to the films for 16 hrs. and then removed for observation as before; the number of crystal clusters per unit area was counted, the area chosen being that covered by the field of a microscope when the plates were moved in one direction from one side to the other. The results are given below (Table VI, fig. 5) and show a clear correlation between crystal size and toxicity, although in the case of ether, crystal size was for some unexplained reason, not related to boiling point.

TABLE VI.

Solvent	Ether	Acetone	Chloro- form	Toluene	Solvent Naphtha	Cyclo- hexone
B.P., °C.	39	56	61	111	140	156
No. of clusters/unit area	18	51	50	38	29	7½
Diameter of crystal clusters, mm.	1.4	0.5	0.5	0.68	0.88	3.5
Insects dead and affected, %	67	19	23	37	53	100

Discussion and Conclusions.

Comparisons of the results obtained from tests on filter paper and glass plates and those given by different solvents reveal the considerable differences in toxicities that arise from the rate of crystallisation. It is not unlikely that a similar difference may exist between large and small benzene hexachloride crystals.

It may be stated that, without taking any special precautions to limit the size of insect used, sufficiently satisfactory replication can be obtained from a small number of tests to render the method applicable to the testing of new or different insecticides, but when the purity of a single insecticide is to be determined a more elaborate method becomes necessary.

From the data relating to dosage-mortality relationships, an indication of the median lethal doses of benzene hexachloride, Chlordane and DDT may be obtained, but conditions of the early tests were not sufficiently controlled for the 90 per cent. lethal doses to be determined with the relatively few insects used. The median

lethal doses, taken from the curves, are 0.0016 mg. per sq. cm. for benzene hexachloride, 0.000375 mg. per sq. cm. for Chlordane, and 0.23 mg. per sq. cm. for DDT, and the 90 per cent. lethal doses for benzene hexachloride and Chlordane appear to be in the region of 0.0022 and 0.00135 mg. per sq. cm., respectively. From this it would be expected that, disregarding the effect of volatility, the dosage of benzene hexachloride sufficient for practical disinfection should be not less than $8\frac{1}{2}$ oz. per 12,000 sq. ft. This is roughly equivalent to $3\frac{1}{2}$ oz. per ton in the case of dry-salted goatskins. Kritzing (1946) found 1 lb. of "Gammexane" a sufficient protection for 12,000 sq. ft. of skins, and Bovingdon and Stock (1947, b) suggest, as the result of large-scale practical trials in Nigeria, 4 oz. per ton. The amount of Chlordane needed for protection would be about half that of benzene hexachloride. It is not possible, from the present work, to estimate the dose of DDT needed to ensure practical control of *Dermestes*, but the few results obtained from the larval tests suggest that, under ordinary conditions of storage, DDT alone will be of negligible value.

The effects of DDT, Chlordane and benzene hexachloride differ in nature as well as in degree of toxicity although each appears to function ultimately as a nerve poison. Benzene hexachloride has a markedly rapid action on adult beetles, whereas that of Chlordane or DDT is slower and cumulative. The reason for the greater resistance of the larvae is not immediately apparent; there are many similar instances of larval resistance in other species but, before an explanation can be found, a more thorough knowledge of insect physiology must be required. The greater resistance of the larvae may not have any practical importance in the protection of skins, since death will merely be postponed for a short period and infestations are more likely to arise from migrating adults than from larvae. On the other hand, it is the larvae, not the adults, which do most of the damage to skins.

Field trials show that the volatility of benzene hexachloride does not militate against its use on hides and skins, since treated skins stored for several months have retained their insecticidal power when baled (Bovingdon and Stock, 1947, b). The possibility arises, however, that under certain adverse conditions of storage, such as may frequently occur in tropical countries or during shipment over long distances, volatility might be an important consideration.

Summary.

Comparative tests have been carried out in the laboratory to assess the relative toxicities of Chlordane, BHC and DDT to the leather beetle, *Dermestes maculatus*. The method used was simple enough for routine industrial purposes when comparisons of new, untried contact insecticides are required, and consists in a measurement of the toxicities of solid films produced by a blood-smear technique on glass photographic plates from standard solutions of insecticide.

Among factors influencing the application of the method, the most important were size of insect and size of crystal clusters as influenced by the volatility of the solvent used to form the film. From LD_{50} measurements, BHC and Chlordane proved, respectively, 140 and 600 times as toxic as DDT to the leather beetle. DDT had comparatively little effect on eggs, larvae or adult beetles.

Acknowledgements.

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References.

- BOVINGDON, H. H. S. & STOCK, G. H. (1947a). Control of hide beetle with "Gammexane" and D.D.T.—*J. int. Soc. Leath. Chem.*, **31**, pp. 115–119.
- BOVINGDON, H. H. S. & STOCK, G. H. (1947b). Nigerian experiments in hide improvement.—*Leath. Tr. Rev.*, **86**, pp. 53–55.
- BRADBURY, F. R., HIGGONS, D. J. & STONEMAN, J. P. (1947). A colorimetric method for the estimation of 2:2-bis(*p*-chlorophenyl)-1:1:1-trichloroethane (D.D.T.).—*J. Soc. chem. Ind., Lond.*, **66**, pp. 65–68.
- BUSVINE, J. R. & BARNES, S. (1947). Observations on mortality among insects exposed to dry insecticidal films.—*Bull. ent. Res.*, **38**, pp. 81–90.
- GNADINGER, C. B. (1936). *Pyrethrum Flowers*. 2nd edn. Minneapolis.
- GNADINGER, C. B. (1945). *Pyrethrum Flowers*, Supplement 1936–1945. Minneapolis.
- HINTON, H. E. (1945). A monograph of the Beetles associated with Stored Products. Vol. 1. London, Brit. Mus.(Nat. Hist.).
- KRITZINGER, C. C. (1946). The use of Gammexane in the hide and skin industry.—*Res. Bull. Leath. Industr. Res. Inst., Grahamstown*, nos. 4 & 5.
- KRITZINGER, C. C. (1947). The use of Gammexane in the hide and skin industry—part II.—*Res. Bull. Leath. Industr. Res. Inst., Grahamstown*, no. 16.
- McINTOSH, A. H. (1946). Relation of crystal size and shape to contact toxicity of D.D.T. suspensions.—*Nature, Lond.*, **158**, p. 417.
- PARKIN, E. A. & GREEN, A. A. (1945). Residual films of D.D.T.—*Nature, Lond.*, **155**, p. 668.
- PARKIN, E. A. & GREEN, A. A. (1947). D.D.T. residual films. I. The persistence and toxicity of deposits from kerosene solutions on wall board.—*Bull. ent. Res.*, **38**, pp. 311–325.
- WEBB, J. E. (1947). A spraying apparatus and testing chamber for investigating the residual action of insecticidal deposits.—*Bull. ent. Res.*, **38**, pp. 209–232.
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THE RELATIONS OF THE COASTAL TSETSE OF KENYA TO THE PLANT COMMUNITIES.

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(Plate X.)

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The investigations described in this paper formed part of a general study of the ecology of *Glossina pallidipes* Aust. carried out at Kilifi on the Kenya coast under the auspices of the Department of Tsetse Research of Tanganyika Territory. The presence of *G. austeni* Newst. and *G. brevipalpis* Newst. enabled observations on these two species to be made also.

A short preliminary reconnaissance of the area was carried out in November and December 1934. Work was started in October 1935 and, except for a period of vacation leave from March to September 1938, was continued until the end of August, 1939.

Description of the Area.

Kilifi (3° 38' S., 39° 51' E.) is situated on the coast of Kenya about 45 miles north of Mombasa, at a point where the coast line, running approximately north and south, meets the north shore of Kilifi creek. Kilifi creek, about eight miles long, is a tidal estuary running at right angles to the coast line.

The area covered by the work was confined to the section of country within a radius of about eight miles from Kilifi township on the north side of the creek. The ground ascends inland for a distance of about three miles and is very flat, and then slopes down, gently at first, and finally more steeply towards the Mjibu valley. This is a shallow and comparatively narrow drainage valley which extends inland for a distance of about four miles. Beyond the valley and running north parallel with the coast for a considerable distance are the Sokoke Hills. These hills attain a height of some 450 feet about eight miles from the shore.



Fig. 1.—Vegetational map of Kilifi showing fly rounds.

Vegetation.

The vegetation between the Mjibu valley and the sea is mainly composed of regenerated types. Many years ago the coastal strip was intensively cultivated by slave labour but has now largely reverted to thicket.

There are three clearly defined types of natural evergreen vegetation at Kilifi; thicket growing on coral rag rock, clump thicket and forest. (The east margin of the forest is composed of savannah type wooding.)

(i) *The coral rag thicket* extends from the coast line to an average distance of about three miles inland. Except for small localised areas of native cultivation and seasonal swamps the thicket extends in an unbroken mass from Kilifi creek to Malindi, 40 miles north. Throughout the area included in the investigation, except for a section at the north end, the thicket is homogeneous and its density is singularly uniform. Owing to the outcropping of coral near the shore the thicket there is less robust than further inland. The transition from the shore to the Kibarani area, which marks the western boundary of the coral rag thicket, is clearly seen for, as the soil becomes deeper, the thicket becomes taller and more robust. The thicket canopy is uniformly dense but the undergrowth is not very dense and by assuming a crouching posture, it is possible to walk almost at will within the thicket.

The dominants are as follows :—

Upper Canopy : *Adansonia digitata*, *Terminalia spinosa*, *Carpodiptera africana*.

Lower Canopy : *Grewia* spp., *Vitex* spp., *Markhamia zanzibarica*, *Lannea Kirkii*, *Teclea trichocarpa*, *Strychnos* spp., *Euclea fruticosa*.

Undergrowth : *Popoaria fornicata*, *Vernonia* sp., *Uvaria leptoclodon*, *Landolphia* spp., *Rhoicissus* spp.

It has been said that an area at the north end of the coral rag thicket differs from the main thicket mass. This difference is noticeable, not so much in the species of vegetation as in the undergrowth which is very much less dense than in the coral rag thicket and in the individual trees which, although on the whole larger, are more widely spaced. Visibility within this thicket is very much greater than within the coral rag thicket. Elephants frequent it in the rains and for that reason it has been named "The Elephant Thicket" (Pl. X, fig. 4).

Adjacent to the elephant thicket and bordering the Malindi road is a relatively small area of recent regeneration. The dominant species in this is a fine-stemmed deciduous *Albizzia*—probably *anthelmintica* (Pl. X, fig. 2).

(ii) *Clump thicket* occupies an extensive area between the coral rag thicket and the forest, and is composed of thicket intersected by open grass glades. The thickets are composed of vegetation identical with that of the coral rag but are more dense. The individual thicket clumps vary greatly in size and shape and the areas of grass land between clumps vary greatly also. At the eastern side of the area the thickets are often of considerable size while further to the west they gradually become smaller and more isolated. A type of clump thicket on the edge of the Mjibu Valley is shown in Pl. X, fig. 1. The width of the glades between thickets at the eastern end of the area is usually not more than 20 yards and frequently very much less.

(iii) *The Sokoke-Arabuko forest* covers a very large area and is mainly forest reserve. It stretches along the Sokoke-Arabuko Hills from Kilifi creek to within a few miles of Malindi 40 miles to the north of Kilifi. The forest is very uniform and, so far as the writer is aware, contains no riverine thicket or anything in its composition resembling coral rag or clump thicket. Certain areas contain relatively dense undergrowth but these areas remain in the writer's opinion essentially forest.

The absence of thicket in the Sokoke-Arabuko forest is supported in correspondence by Commander Templar, Forester-in-Charge.

The dominants are as follows :—

Upper Canopy : *Brachystegia spiciformis*, *Isobertinia magnistipulata*, *Mimusops* sp., *Azelia quanzensis*, *Combretum Shumanii*, *Cassipourea euryoides*, *Cynometra* sp., *Trachylobium verrucosum*.

Lower Canopy : *Vitex* spp., *Strychnos* spp., *Phyllanthus* spp., *Lannea* spp.

Undergrowth : *Landolphia* spp., *Canthium* spp., *Teclea fruticosa*, *Uvaria leptoclodon*, *Clerodendron* spp.

(iv) *Savannah*.—This term has been given to the eastern edge of the Sokoke forest where it borders the Mjibu valley. The general aspect of this marginal wooding resembles *Isobertinia-Brachystegia* savannah in that both these genera are present (and dominant) and that the trees are more widely spaced than is usual in coastal forest. There has been much cutting out of timber and to this factor more than to any other the area owes its savannah-like appearance.

(v) *Cultivated areas*.—There is much European cultivation as well as native cultivation at Kilifi. The European cultivation is situated in the centre of the area included in this investigation and comprises a large acreage in the locality known as Kibarani (Plate X, fig. 1).

About a mile north of the European cultivation is an extensive area under native settlement and cultivation ; the crops are varied and much of the land is taken up by coconut palms and bananas. Scattered about the countryside are numerous settlements wherein the natives follow their ancient practice of crop and thicket rotation. It is usual for the natives to put partially cleared land under crops for three years after which time the thicket is allowed to regenerate for 10 to 15 years.

Climate.—The climate of Kilifi may be divided into three seasons :—

- (a) The wet season : April to June or May to July, depending whether the rains come early or late ;
- (b) The humid season : July or August to October ;
- (c) The dry season : November to March, though this may be broken by a wet spell in December.

The total rainfall registered at the Standard Meteorological Station at Kibarani varied from 34.8 to 48.7 ins. during the period 1931–37, the average over the seven years being 41.9 ins. February was the only month completely without rain, and that only in three years out of the seven, its average being over three-quarters of an inch ; the other months all averaged more than an inch, and only January and March fell below this figure. May shows the highest rainfall, averaging over 11 ins., with a maximum of 15.7 in 1935.

The mean monthly minimum temperatures during the years 1936,7 ranged between 20.3°C. in August 1936 and 25.0°C. in April 1936, whilst the maxima varied from 27.8°C. in July 1936 to 33.1°C. in February and March 1937. The mean monthly relative humidity measured at 2.30 p.m. never fell below 63 per cent., the figures varying between that and 70 per cent. in the dry season (except for 76 per cent. in December 1937—an instance of a wet spell in that month) ; in the wet and humid seasons the mean monthly figures lay between 70 and 80 per cent.

Measurements in the Coral Rag Thicket (taken under canopy) showed that the temperatures in that type of habitat did not differ to any great extent from those of the Standard Station in the open, though the relative humidities taken at 8.15 a.m. could exceed by as much as 10 per cent. the monthly means of the Standard Station, taken 15 minutes later. This habitat comprises the thickest vegetation studied and is therefore the most likely of any to show differences.

Animals.

All vegetation communities at Kilifi support a large population of small animals. There is little difference in the components of the populations of the various vegetation types. The only animals that are truly migratory are elephants. Herds of elephants enter the northern end of the area from the Malindi District about the middle of May and stay in the vicinity until about the middle of August, that is to say, they are present in the rainy season. Owing to protective measures they have been confined in recent years to the northern end of the area. It is only occasionally that raids are made by elephants in the vicinity of Kilifi. Buffalo are always present in the area both in the coral rag thicket and in the Sokoke forest. During the rains they appear to be increased by herds migrating from the north.

Waterbuck were recorded in the marginal forest in January and February when their visits were nocturnal.

An increase in the number of animals present in the thicketed areas was recorded during the humid season, while in the savannah and in the Sokoke forest an increase was recorded in the dry season.

The density of game in all vegetation types was about the same, but a preference was shown by the animals for the lighter types of thicket and forest.

Birds and snakes were very numerous.

The following Ungulates and Primates have been identified by Dr. van Someren :—

Dikdik (*Rhynchotragus kirki nyikae*), red duiker (*Sylvicapra natalensis harveyi*), duiker (*Sylvicapra grimmia deserti*), coast blue duiker (*Cephalophus monticola hecki*), white leg duiker (*Cephalophus nr. adersi*), bushbuck (*Tragelaphus scriptus olivaceus*), waterbuck (*Kobus ellipsiprymnus*), bush-pig (*Potamochoerus koiropotamus daemonis*), wart-hog (*Phacochoerus aethiopicus*), grey monkey (*Lasiopyga pygerrhina contigua*), red rumped monkey (*Lasiopyga albogularis maritima*), baboon (*Papio cynocephalus ?*), lemur (*Galago crassicaudatus*) ; a number of the carnivores, including the hyena (*Crocuta* sp.) and the hunting dog (*Lycan pictus*) ; rodents from this area were also identified, and monitor lizards (*Varanus* sp.) also occurred.

DISTRIBUTION OF THE VARIOUS SPECIES OF TSETSE IN THE DIFFERENT VEGETATION COMMUNITIES.

Glossina pallidipes.

General distribution.

G. pallidipes of both sexes were evenly distributed throughout all homogeneous vegetation communities. A long fly round was specially devised in order to ascertain whether tsetse were present in the centre of the coral rag thicket remote from settlement, cultivation and roads. This round was carried out once a week only ; it was purposely not made more often in order to ensure that there could be absolutely no possibility of tsetse concentrating along the fly round path waiting for the party. All tsetse were marked on this round and were not recaptured further along it. Examination of the data from this round which was made twenty-two times between May and October 1936 shows that many *G. pallidipes* were taken on the sections which penetrated deepest into the thicket.

The coral rag thicket round was well placed for the study of the distribution of tsetse within the thicket. The round penetrated deeply into homogeneous thicket which, at the time the round was being made, was reasonably remote from settlement and cultivation. The distribution of tsetse per 500 yards, for the morning catches in both the humid and the dry season, for the transverse sections running north and

TABLE I.
Morning catches.—Coral Rag thicket round.
Distribution of *G. pallidipes* on transverse sections.

Section No.	Tsetse per 500 yds.		Female per cent.		Hunger	
	Season		Season		Season	
	Humid	Dry	Humid	Dry	Humid	Dry
1 and 2...	2	5	18.8	43.9	3.32	3.14
4 " 5...	9	5	27.9	40.3	3.04	3.12
7 " 8...	14	5	26.7	26.9	3.07	3.05
10 " 11...	13	8	33.6	29.8	3.02	3.05
13 " 14...	17	8	38.6	33.9	3.14	3.07
16 " 17...	14	11	39.7	31.7	3.04	3.04
19 " 20...	9	12	42.9	36.1	3.05	3.10
22 " 23 (road)...	12	7	41.5	43.9	3.30	3.18
6, 12 and 18 (road)	9	3	33.5	41.0	3.13	3.17
Means	11	7	33.7	36.4	3.12	3.30

All fly-rounds were made by African fly-boys accompanied by two docile bait cattle. With few exceptions the writer was a member of the party on all fly-rounds.

south is given below. The road sections have been recorded at the bottom of the Table. Sections Nos. 1 and 2 were the most easterly sections running close to and parallel with the shore. Sections Nos. 19 and 20 were the most westerly sections running parallel with the Malindi road which formed sections Nos. 22 and 23.

It will be seen from Table I that at all seasons the centre of the thicket was as densely populated as the external sections and, indeed, in the humid season there were more tsetse per 500 yards in the centre of the thicket, which was over 1,500 yards from the external sections on the Malindi road and the sea shore, than elsewhere.

The Sokoke forest round, which penetrated deeply into forest reserve, furnished information on the distribution of *G. pallidipes* in homogeneous vegetation, for the greater part of the round was far removed from settlement and cultivation.

TABLE II.
Morning catches—Sokoke Forest round.
Distribution of *G. pallidipes*.

Section No.	Tsetse per 500 yds.		Female per cent.		Hunger	
	Season		Season		Season	
Nature	Humid	Dry	Humid	Dry	Humid	Dry
1. Marginal forest	7	6	46.4	29.3	3.06	3.08
2. Marginal ...	8	14	41.3	27.9	3.10	3.07
3. Marginal forest	10	3	47.7	38.6	3.05	3.01
4. Marginal ...	16	7	49.6	38.1	3.05	3.17
5. Forest...	21	10	57.1	42.6	3.06	3.08
6. Open ...	5	3	40.6	30.6	3.02	3.04
7. Forest...	7	5	53.1	50.1	3.04	3.03
8. Old cult. ...	4	3	47.1	24.8	3.10	3.04
9. Road ...	5	4	30.8	20.0	3.04	3.03
10. Forest...	7	10	43.3	51.0	3.13	3.06
11. Forest...	8	13	56.3	41.3	3.08	3.11
12. Forest...	6	9	46.8	42.3	3.06	3.06
13. Forest...	5	6	41.8	40.3	3.16	3.06
14. Road ...	2	2	27.4	22.7	3.14	3.09
Means ...	8	7	44.9	35.7	3.07	3.07

A typical piece of forest was that which was penetrated for a distance of 1,300 yards by section No. 5. This section passed from a large seasonal swamp eastwards and was far removed from settlement and cultivation; it was not frequented by natives. It will be seen from Table II that *G. pallidipes* was taken in large numbers on almost all sections of the round but that, on average, throughout the year the greatest density was to be found on section No. 5, and in this area also was to be found the highest proportion of female tsetses. Sections Nos. 10 to 13 passed through forest remote from any other vegetational contact and the tsetse population in that area compared favourably with other areas.

The tsetses were distributed evenly throughout the surrounding savannah (Table III). The greatest density-activity was encountered on section No. 2 which was the section most remote from marginal contact with the Mjibu valley.

It is not possible to consider the distribution of *G. pallidipes* through the clump thicket area without bearing in mind the factor of insolation which brought about small catches in the open sections. The greatest density of tsetses was encountered at the eastern end of the area in the proximity of the coral rag thicket but the thickets remote from the coral rag also supported a large population.

TABLE III.

Morning catches, Savannah round. Distribution of *G. pallidipes*.

Section No.	Tsetses per 500 yds.	Female %	Hunger	Nature of section.
1	23	43.0	3.12	Native path through <i>Brachystegia</i> wooding.
2	46	40.5	3.05	<i>Brachystegia</i> wooding.
3	14	28.6	3.11	Open glade and light woodland.
4	22	34.5	3.05	Edge of marginal forest and Mjibu valley.
5	62	37.3	3.05	Isthmus of marginal forest across valley.
6	29	39.0	3.09	Edge of marginal forest and Mjibu valley.
7	23	38.8	3.05	Mixed <i>Lannea-Vitex-Brachystegia</i> wooding.
8	21	29.8	3.20	Mixed mainly open with clump thicket.
9	20	29.0	3.20	Poor <i>Grewia</i> , <i>Vitex</i> , <i>Dichrostachys</i> spp.
10	6	32.2	3.15	Road, <i>Azelia-Mimulus-Brachystegia</i> wooding.

The distribution of *G. pallidipes* in the elephant thicket was influenced very greatly by the presence along its western edge of a strip of recent regeneration. This strip was approximately 300 yards deep on the eastern side of the Malindi road and fronted each side of the road north from Mtondia village for a distance of about one mile. There was within this recent regeneration a tremendous concentration of tsetses which spread back into the elephant thicket for a distance of about 300 yards. The average density-activity figure per 500 yards for the seven remaining sections of the round was ten tsetses over the whole year. On the section of elephant thicket behind the recent regeneration the average density-activity figure per 500 yards for the year was 25 tsetses, while that for the recent regeneration itself was 50 tsetses per 500 yards.

Distribution in marginal areas.

The following types of marginal section are discussed below :—

- (i) Road marginal sections in which a possible motor road provided a cleared space through vegetation which would otherwise be continuous.
- (ii) External marginal sections along the line of demarcation between two clearly defined vegetation communities.
- (iii) Internal marginal sections such as those which passed round the edges of seasonal swamps and waterholes within homogeneous vegetation (Pl. X, fig. 4).
- (iv) Broken marginal sections such as those formed by clump thickets with open grass-covered glades between thicket clumps (Pl. X, fig. 1).
- (v) Terminal sections where land met sea.

The paths on all rounds passed within a few feet of the edge of the vegetation type which they were designed to tap.

Road marginal sections.—No concentrations of *G. pallidipes* were noted on this type of section. Density-activity conformed to that within the vegetation contiguous with the roads.

External marginal sections.—No concentrations of *G. pallidipes* were found on these sections. Section No. 5 of the savannah round passed through the centre of the long axis of isthmus of wooding running across the Mjibu valley. In this section, as may be seen in Table III, the density-activity was high. In contrast the external marginal sections Nos. 4 and 6 which were adjacent to this section showed a relatively low density-activity.

Internal marginal sections.—This type of section on the Sokoke forest round showed contradictory results. A concentration appears to have been present during the dry

season on the edge of the small seasonal swamp tapped by section No. 2 (Table II). During that season no concentration was noted along the edge of the large seasonal swamp tapped by section No. 4 of the round although, during the wet season when the concentration on section No. 2 disappeared, a concentration appears to have formed there.

A catch taking place on the elephant thicket round during the wet season at a time when a large herd of elephants was in the habit of drinking at the pool is shown on Pl. X, fig. 3. The pool was situated a little further down the right-hand edge of the "mbuga" shown in Pl. X, fig. 4. On this section of the round which was made from February 1937 to the end of February 1938 no concentration of *G. pallidipes* was at any time recorded.

Broken marginal sections.—This type of marginal section was traversed by the clump thicket round. On those sections which passed through the larger clumps the density-activity was at all seasons greater than that recorded on sections which passed close to the edge of clump thickets or traversed the open glades between them.

Terminal sections.—The only examples of this type of section were Nos. 1 and 2 on the coral rag thicket round. From Table I it will be seen that no concentration was observed on these sections.

Distribution in coconut plantation.

In the top left-hand corner of the map (fig. 1) is a large unshaded area marked "Sokoke". This unshaded area shows the position of the Sokoke coconut plantation covering an area of 1,800 acres in which the now fully grown trees are planted in orchard fashion at intervals of eight yards. The undergrowth is kept down to economical proportions and fallen fronds are piled between rows. There is at all times considerable native traffic through the plantation and cars and lorries frequently pass between Kilifi and the plantation factory. The plantation is surrounded by forest on three sides and on the west side by regeneration. It will be noticed from the map that the Sokoke forest fly round passed through the forest to the east of the plantation and from data collected on this round it is known that the forest abutting on the plantation is heavily infested with tsetse of all three species. Tsetse are also known to be present in the regeneration on the west side of the plantation in the wet season.

In order to ascertain to what extent tsetse spread into the plantation three catches with a bait animal were made in June 1939 in the south-east corner; they followed a constant route and covered an area bounded by the road running along the south boundary and up the centre of the plantation as far as the factory (shown as a square on the map) and thence to the forest on the east side. This area was about 1,000 yards square. The final course of the catch passed for 1,000 yards at a distance of 35 yards from the forest edge. The catches started at 6.15 a.m. and lasted for about two hours and resulted in a total of four *G. pallidipes* and one *G. austeni* being taken in the plantation. While passing along the forest edge a total of three *G. pallidipes* and one *G. brevipalpis* was recorded.

During July four catches were made in the plantation starting at 8 a.m. lasting for about 90 mins. each. During these catches the whole of the plantation was searched for tsetse. One female *G. pallidipes* was the only tsetse captured; it was taken close to the main road and near a group of native huts. Conditions during these searches, which were made with the aid of a bait animal, were very suitable for tsetse activity.

It is considered that, except for three months in the year, during the wet season, the plantation will be virtually tsetse-free.

Seasonal distribution.

The study of the seasonal distribution of *G. pallidipes* was complicated by "rush activity" in some vegetation types.

Note.—Explanation of the term "rush activity"—

Rush activity is a term coined by the writer to indicate the violent early morning positive reaction of *G. austeni* and, to a lesser extent, *G. pallidipes* to dry atmospheric conditions and is brought about by the need to find a host before adverse physical conditions cause the tsetse to become inactive. The failure of the majority of a population of *G. austeni* to find a host results in much increased activity at night in a continuation of the search under the relatively cool and very humid conditions then prevailing.

On the coral rag thicket round there is reason to believe that the monthly density-activity figures do represent actual changes in density and that the factor of rush activity was absent. The average humid season density was 1.6 times the dry season density. The difference was most apparent in the eastern half of the coral rag thicket where the vegetation was less robust than in the western half. During the dry season, density in both halves of the thicket decreased but, whereas there was a decrease of 50 per cent. in the eastern half, in the western half the decrease amounted to only 30 per cent. It would seem that as the seasonal fluctuations in density were greatest in the eastern half of the thicket, a seasonal spread took place from the denser vegetation in the humid season and back to it from the lighter vegetation in the dry season.

Throughout the whole of the clump thicket round, a very marked humid season increase was noted and, as this round (like the coral rag) was free from the influence of rush activity, the increase in the density-activity figures amounting to nearly threefold may be accepted as representing, in part, a genuine increase in true density. In view of the increase that took place during the humid season in the coral rag thicket itself, it cannot be to any seasonal spread from there that the clump thicket owes in the main its increase in density. That a genuine increase in density did take place in the clump thickets cannot be doubted, but that the seasonal activity of the tsetse played a large part in the increased catches seems to be borne out by a study of the data. The largest increases on individual sections took place on open sections and it is clear that during the humid season *G. pallidipes* attacked across the narrow grass glades more readily than it did during the dry season, so that the increase in the catches represents an actual increase in density of the tsetse accompanied by an increased willingness to attack.

Glossina austeni.*General distribution.*

This tsetse was found in all vegetation types at Kilifi. In the Sokoke forest *G. austeni* was taken in numbers approximating to those of *G. pallidipes*, but elsewhere in small numbers only. On the coral rag thicket round and on the savannah round a marked preference was shown for areas supporting denser vegetation but, in the case of the latter round, internal sections showed greater density than marginal sections and seasonal increases were confined almost entirely to internal sections. The distribution of *G. austeni* was very even throughout the Sokoke forest area except on road sections and on a section that passed through an area of abandoned cultivation from which tsetse were almost entirely absent.

Distribution in marginal areas.

The figures for the individual catches of *G. austeni* on most rounds were very small and it is difficult to form an opinion on the distribution of this species along the edge of marginal sections. On the coral rag thicket round the evidence is contradictory for, on the sections along the narrow private road leading from the shore to the Malindi road, the tsetse were recorded in smaller numbers than elsewhere on the round. On the other hand the catches made on the two sections on the Malindi road showed, that, although the tsetse did not appear to be attracted to the sections during the humid season, they were taken in considerable numbers during the dry season. The greater numbers of tsetse of both sexes captured on the road during the dry season suggests that the tsetse may have been concentrating along the road. During the humid season the percentage of females captured on the Malindi road was only 17.7 but during the dry season the percentage increased to 50.5. Road sections on the Soko forest round, however, lend no support to the evidence of seasonal concentration such as was suggested by the data from the Malindi road. On both sections Nos. 9 and 14 on the Soko forest round *G. austeni* was taken at all times in very small numbers and the percentage of females taken on the long road section No. 14 averaged only 13 for the two seasons. On the Malindi road sections of the coral rag thicket round the female percentage on the dry season catches had been very high and those for the humid season low. On the Soko forest round road section No. 9, however, the dry season female percentage was nil while that for the humid season was 65.5. The only other evidence of concentration along a marginal section was found on section No. 2 of the Soko forest round. The concentration suggested by the figures for the catches made on this section was confined to the dry season only and, in any case, the proportion of tsetse captured was not greatly in excess of those captured on other sections of the round. The large seasonal swamp which was tapped by section No. 4 of the Soko forest round showed no evidence at all of any concentration either during the humid or the dry season, nor did the marginal sections along the Mjibu valley on the savannah round. On the elephant thicket round *G. austeni* was very scarce but along the margin of the "mbuga" the tsetse were at all times almost completely absent (Pl. X, fig. 4). When such factors as rush activity and vegetation preferences have been taken into account it is thought that the balance of evidence shows that *G. austeni* did not at any season concentrate on marginal sections.

Seasonal distribution.

The data have been searched for evidence of a wet season spread of *G. austeni* from the areas of heavier vegetation into areas of lighter vegetation. The coral rag thicket furnished the best example of the two types as the eastern half of the area tapped by the round was more lightly thicketed than the western half. Throughout the year about 75 per cent. of the *G. austeni* taken were captured in the western half of the thicket. During the wet season there was no evidence of a spread of these tsetse from the western half of the thicket to the eastern half; indeed, 14 per cent. more tsetse were captured in the eastern half of the thicket in the dry season as compared with the humid season. This small dry season increase may be attributed to the influence of rush activity. On the savannah round the proportion of tsetse captured on internal sections in the humid season did not vary and there was no increase at this season on marginal sections that did not take place also on internal sections. On the Soko forest round there was very little change indeed in the numbers of tsetse taken on individual sections at any season of the year. The exception is furnished by section No. 7 which contained much close-growing recent regeneration. On this section, an average of 19 *G. austeni* were taken over 500 yards in 40 catches during the humid season, but in the dry season the average was only one in the course of 22 catches.

Glossina brevipalpis.

Except at dusk, catches of this crepuscular tsetse were confined to single individuals at wide intervals. It is natural that the terminal sections of all rounds when made in the evening should have produced more *G. brevipalpis* than other sections. Under these circumstances it is not possible to express an opinion on the distribution of this species and its seasonal changes of habitat. An inspection of the data from sections other than terminal sections shows that the tsetses were taken in small numbers evenly throughout the Soko forest rounds both in the morning and afternoon, but that they were nearly absent from marginal sections and open sections on morning catches and from the edge of the large seasonal swamp and the open section No. 6 of the forest round on afternoon catches. The afternoon round finished at section No. 9, but comparing the morning and afternoon catches over the nine sections the afternoon catch in the humid season produced three times as many tsetses as the morning catch and the dry season afternoon catch produced 75 per cent. more tsetses than the morning catch. The difference in numbers captured on afternoon rounds in the two seasons was, on average, one tsetse only per 500 yards.

The female percentages of the catches were too low to be accepted as furnishing reliable data.

The afternoon catches on the coral rag thicket round made in the humid season only, showed that more than one and a half times more tsetses were taken in the western half of the thicket, a large proportion of which were taken on the transverse sections, Nos. 16 and 17. The thicket on these two sections was not particularly dense and there were open spaces in which undergrowth was scanty and visibility comparatively good. The catches made on sections Nos. 19 and 20 showed that the tsetses apparently did not share with *G. austeni* a preference for the heavy wooding of these two sections.

Special Distribution Experiments.

These observations, based on the accumulated data from routine fly rounds, lead to the conclusion that *G. pallidipes* is evenly dispersed throughout all the vegetation types studied. This conclusion is somewhat at variance with that reached by Swynnerton (1933) who sums up his own and other investigators' experience by saying that "when thicket is really dense and *continuous* the italics are mine] *G. pallidipes* seems likely to frequent mainly the outskirts." In view of this it was considered desirable to confirm the observations by direct experiments and these were accordingly carried out in January 1939.

A narrow path was cut through the coral rag thicket in an easterly direction for a distance of 800 yards from the Malindi road at a point about 200 yards north of the junction of the long fly round with the coral rag thicket fly round (see map, fig. 1). The path then turned north-east into the heart of the thicket for a distance of half a mile. This path was very twisty and inconspicuous. To the north of the terminus of the path there was cut a path which formed a rectangle of which the dimensions were 100 yards by 250 yards. Within this rectangle the thicket was untouched.

Two catching parties, each with a bait animal, assembled at the rectangle before daybreak. Movement through the thicket to the rectangle before daybreak attracted no *G. pallidipes*, but it was realised that when the tsetses became active after sunrise they might follow the scent of the parties and so arrive at the rectangle to be captured and recorded as having been located at the rectangle. In order to meet this criticism the following method was used: When catching started at sunrise one party caught round the rectangle while the other party patrolled from the rectangle back along the path leading to it. The purpose of this latter party was to intercept any tsetses

following the scent of the party along the path. In this way only tsetses living in the vicinity of the rectangle were captured there. Four of these catches were made. The mean catches of *G. pallidipes* and *G. austeni* made around the rectangle and along the path for a distance of 200 yards from the rectangle are given in Table IV.

TABLE IV.

Catches made around a rectangle and along a path in the centre of the coral rag thicket.

Rectangle.

Species	Rate per hour	Female per cent.
<i>G. pallidipes</i>	96	39.1
<i>G. austeni</i>	19	45.7
<i>G. brevipalpis</i> (total) ...	24	37.5

Path.

Species	Rate per hour	Female per cent.
<i>G. pallidipes</i>	35	36.3
<i>G. austeni</i>	6	46.5
<i>G. brevipalpis</i> (total) ...	6	66.6

It should be realised that catching started at dawn and that *G. pallidipes* was not fully active until at least half an hour after the catches had commenced.

Immediately after the rectangle had been set out a search for pupae was undertaken within the rectangle. The search which lasted about 74 boy-hours resulted in the finding of pupae and cases as under :—

Species	Pupae	Cases
<i>G. pallidipes</i>	1	11
<i>G. austeni</i>	17	480
<i>G. brevipalpis</i>	0	2

These catches, coupled with the findings of pupae in the rectangle, prove beyond question the presence of all three species in the centre of the thicket.

Hunger.

Jackson (1933) worked out a system whereby tsetse-flies may be divided into four stages of hunger by the external appearance of the abdomen, *viz.* : Stage I, gorged ; Stage II, replete ; Stage III, intermediate ; Stage IV, hungry. By a simple calculation the Mean Hunger Stage (M.H.S.) of a sample of tsetses may be determined. The calculation is worked to two places of decimals. Stage I being excluded, a state mid-way between satiety and hunger would receive the value 3.00. Figures having a value higher than 3.00 indicate that the tsetses in the sample are on average more hungry than replete. Values lower than 3.00 indicate a state of repletion rather than hunger. The degree with which the values vary about the mean will be influenced by the degree of repletion or hunger in the sample. Females and young tsetses are not included in the calculation.

All male *G. pallidipes* were examined and staged for hunger but the examination of *G. austeni* was confined to the Sokoke forest round as the samples from the other rounds were too small to give reliable data. No attempt was made to stage the hunger of *G. brevipalpis*, but it cannot be recalled that in any individual captured there were indications of real hunger.

*G. pallidipes.**Seasonal hunger.*

The population at Kilifi was at all times replete. On the coral rag thicket round, the savannah forest round, the elephant thicket round and the Sokoke forest round the mean hunger stage was higher in the humid than in the dry season. It will be seen from Tables I and II that the difference in hunger between the two seasons was very slight. The clump thicket round, which differed from the other rounds in that it was not made through homogeneous vegetation, showed that the tsetsees there were hungrier in the dry than in the humid season. Afternoon rounds showed slightly higher mean hunger figures than morning rounds. There was at all times and in all vegetation communities a sufficiency of animals for the tsetse population. During the dry season the undergrowth and especially the woody plants and grasses died back and visibility was, as a result, greater in the dry season than in the humid season. The majority of animals were small and visibility at low levels would influence the ability of the tsetsees to make contact with their hosts. During the humid season hunting would be rendered more difficult on account of limited visibility and this factor was reflected in the slightly higher mean hunger stage of the males of the population at that season. The higher hunger which was noted in the dry season in the clump thickets may have been due to an evacuation of the clumps by the game during the dry season. It is more probable, however, that the movement of hungry tsetsees was restricted by unfavourable conditions prevailing outside the thickets, confining the passage of tsetsees from one thicket clump to another to the early hours of the morning, so that they did not have so good a chance of getting food.

Distribution of hungry tsetsees.

The sections on which hungry tsetsees were recorded were confined almost exclusively to road sections and to the humid season. The absence of game in marginal areas, where the atmosphere is drier, would tend to increase hunger but the number of tsetsees found in this predicament was very small. The exception was the road section of the elephant thicket round. On this section exceptionally high hunger was associated with large catches in the humid season. During the dry season, at the time of rush activity, higher catches were made than even in the humid season but the mean hunger stage was low. The conditions influencing the high catches on the Malindi road section of this round were exceptional and could be attributed to a tremendous concentration of tsetsees within a narrow belt of recent regeneration in close proximity to the road. The higher hunger stage in the humid season is of interest for there was an increase in the number of head of game reported and elephant were present. These conditions lend support to the theory that limited visibility was the major factor influencing greater hunger during the humid season.

On internal marginal sections such as the seasonal swamp near the Mtondia cultivation, the small and large Sokoke forest seasonal swamps, the edge of the Mjibu Valley and the elephant thicket "mbuga" there was no indication of the presence of hungry tsetsees as judged by the standards of other tsetse communities and by those of the road sections of the Kilifi area.

G. austeni.

The mean hunger stage of the male population in the Sokoke forest was 3.02 on morning rounds and 3.00 on afternoon rounds. There is nothing in the data to suggest any seasonal intensity of hunger or any definite distribution of hungry tsetsees. The percentage of male tsetsees which showed black blood in the abdomen was 22.3 on the morning catches and 18.2 on the afternoon catches. The percentage of tsetsees containing black blood varied from 42, which was the average for the afternoon catches during August, to seven during January. The range in the morning catches varied from 34 per cent. in March to 14 per cent. in December. This proportion of

male tsetse showing black blood in the abdomen is very large in comparison with the numbers of *G. pallidipes* taken in the same condition. In the Sokoke forest the percentage of male *G. pallidipes* showing black blood was 0.8. In view of the large percentage of male *G. austeni* captured, showing evidence of a recent meal, it is not surprising that the mean hunger stage of the population as a whole was so very low. It is surprising that the replete males should be active in the vicinity of the party.

Pupal Habitats.

Pupa searches were undertaken in all vegetation communities for a period extending over six months and it is estimated that about 360 boy-hours were spent on this work. Searches were made in classical sites and on the floor of the thicket and forest. *G. pallidipes* apparently deposited its larva indiscriminately on the floor of the thicket or the forest and showed no preference for classical sites although pupae were found in them. The numbers of pupae and pupal cases of *G. brevipalpis* found were very small but, so far as could be judged, this fly appeared to have a preference for logs and the bases of thickets. The pupal cases of *G. austeni* were found readily in large numbers in all vegetation communities and the species gave indications of a very marked preference for the underside of large logs. In the clump thicket area, where *G. austeni* were not taken in great numbers, large finds of over 100 pupal cases were made under individual rotting logs.

The total numbers of pupae and pupal cases found in the course of the searches are shown in Table V.

TABLE V.
Pupae and pupal cases found.

Totals		<i>G. austeni</i>	<i>G. pallidipes</i>	<i>G. brevipalpis</i>
Cases		2,147	169	33
Pupae		133	11	8

A similar preponderance of the pupae and cases of *G. austeni* was recorded by the writer when working at Kiwanda near Amani in Tanganyika.

Summary.

Under the conditions prevailing at Kilifi *G. pallidipes* is evenly distributed throughout all vegetation types with the exception of cultivation from which it is at all times almost entirely absent. There is a preference for the denser vegetation in the dry season from which the tsetse spread out into the lighter vegetation in the wet and humid seasons. There is never at any time any concentration of *G. pallidipes* along the edges of extensive thicket or forest.

The distribution of *G. austeni* is similar to that of *G. pallidipes* except that there is no tendency to spread into lighter vegetation in the wet season.

There is some reason to think that *G. brevipalpis* prefers thicket of medium density and does not share with *G. austeni* a preference for tall heavy thicket.

G. pallidipes was the only species staged for hunger, except for *austeni* in the Sokoke forest. Males are replete at all seasons. *G. austeni* showed a remarkably large proportion of recently fed males.

Pupae and pupal cases of *G. pallidipes* were found on the floor of the coral rag thicket and forest. *G. austeni* and *G. brevipalpis* showed a preference for the undersides of large logs.

Acknowledgements.

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To Mr. P. Greenway, Botanist, Amani Agricultural Research Station, grateful acknowledgment is made for his great assistance in identifying the majority of plants collected.

Thanks are offered to the Game Warden, Kenya Colony, and to Dr. van Someren, formerly Curator of the Coryndon Memorial Museum, Nairobi, for their assistance in identifying the skins of animals sent in to them.

It was due to the kindness of Squadron-Leader Taaffe that the aerial photographs shown in this paper were taken and a flight in an R.A.F. Machine made over the Kilifi area. This kindness is gratefully acknowledged. Figures 1 and 4 of Pl. X are reproduced by kind permission of "Air Ministry Crown Copyright".

The writer is greatly indebted to Mr. Napier Bax, Acting Director, and to Mr. W. H. Potts, Senior Entomologist, and Dr. C. H. N. Jackson, Department of Tsetse Research, for reading through the manuscript and for making many valuable suggestions and criticisms.

It would appear ungrateful indeed were a word of praise not given to the native staff. Throughout the investigation the work was well and cheerfully performed and for this the head boy, Maganga Malale, was largely responsible. The herd boys deserve special mention for on no occasion were they late with the cattle, either day or night, at the start of the many fly rounds.

References.

- JACKSON, C. H. N. (1933). The causes and implications of hunger in Tsetse-flies.—
" Bull. ent. Res., **24**, pp. 443-482.
SWYNNERTON, C. F. M. (1936). The Tsetse Flies of East Africa.—Trans. R. ent.
Soc. Lond., **84**, pp. 1-579.
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FIG. 1. Clump thicket in Mijibu Valley. The Kilifi-Soko road is visible in the top right-hand corner of the photograph, and a portion of section 8 of the savannah round, where it passes between the thicket clumps growing at the edge of a large seasonal swamp, in the lower right-hand corner. The European cultivation of Kibarani is to be seen in the top left-hand corner.



FIG. 2. Edge of recent regeneration of the elephant thicket (Section 1 of elephant thicket round).



FIG. 3. Fly catching party on edge of pool on section 3 of the elephant thicket round shown in figure 4.

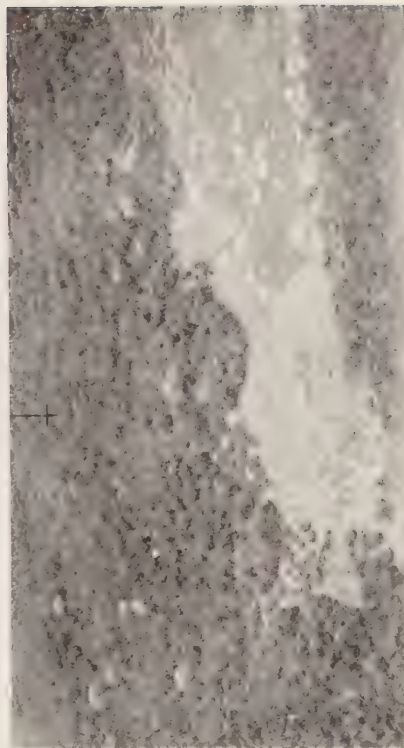


FIG. 4. Nibuga surrounded by elephant thicket.

TWO NEW AFRICAN INJURIOUS GRASSHOPPERS.

By V. M. DIRSH, D.Sc.

*Anti-Locust Research Centre.****Eyprepocnemis noxius* sp. n.** (Figs. 2 & 4c).

♂ (*Type*). Antennae somewhat longer than the head and pronotum together. Frontal ridge flat, punctured, gradually narrowed to the apex, its margins obtuse. Fastigium of the vertex slightly concave, oval, with rounded apex and almost smoothed lateral carinulae. Occiput with a scarcely distinguishable median carinula.

Pronotum above flat. Median carina in profile straight; lateral carinae slightly excurved, smoothed in the posterior part of the metazona; prozona slightly longer than metazona, its anterior margin truncate; posterior margin of metazona broadly rounded. Lateral lobe of the pronotum with projected lower margin. Prosternal tubercle subcylindrical, inclined backward, its apex slightly narrowed and rounded.

Elytron well developed, projecting beyond the hind knee, its apex broadly rounded. Wing broad, with wide apical part.

Subgenital plate short, subconical, its upper margin, in profile, almost straight, the lower convex. Supra-anal plate oval, with slightly projecting apex. The cerci project beyond the apex of the supra-anal plate and at the base are robust and broad, in the apical part narrowed, compressed and slightly decurved; the apex obliquely truncate.

Colouration brown; sides of the pronotal disc greyish-yellow. Elytron with numerous small, dark spots and with the yellow stripe along the ulnar vein. Wing light yellowish at the base, colourless in the rest, with blackish reticulation. Hind femur on the external side dark, above and inside with two indistinct fasciae; upper lobe of the hind knee dark. Hind tibia uniformly pink-violet; tibial spines at the base pale with dark almost black, apical third.

Measurements: Length of body ♂ 26-30 (*Type* 26), ♀ 31-40; pronotum ♂ 5-6 (*Type* 6), ♀ 6-7.5; elytron ♂ 23-28 (*Type* 24.5), ♀ 27-36; hind femur ♂ 15.5-17.5 (*Type* 15.5), ♀ 18-22.

ANGLO-EGYPTIAN SUDAN: Gedaref, iv-xii, 1946, 15 ♂♂, 5 ♀♀. Plains 30 miles west of Gedaref, 24.viii.1948, 1 ♂ (*Type*), x.1948, 3 ♂♂, 5 ♀♀ (*R. J. T. Joyce*). Wadi Showil, 11.viii.1933, 1 ♂ (*R. C. Maxwell Darling*).

TANGANYIKA TERRITORY: Old Shinyanga, 2.xi.1935, 1 ♀; 2.vi.1938, 1 ♀ (*E. Burtt*).

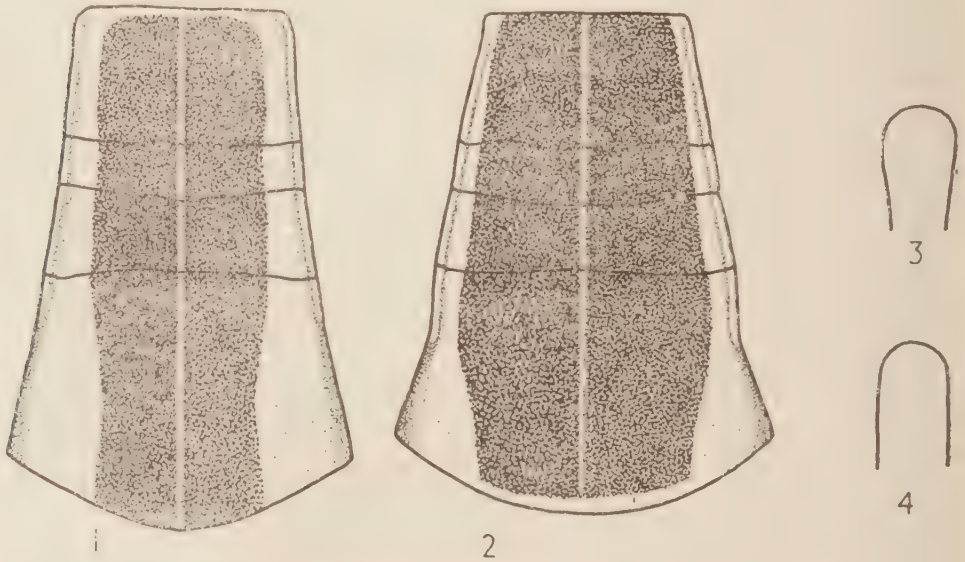
NIGERIA: Kalkala, vi-x.1934, 4 ♂♂, 7 ♀♀ (*A. M. Gwynn*).

General colouration in specimens of *E. noxius* varies from light yellow-ochraceous to dark brown and greyish; lateral stripes of the pronotum vary from light yellow to dirty yellow or greyish and brownish. Hind tibia varies from light to dark reddish-violet, but about half of all specimens have a light blue hind tibia and there are no intermediates between the blue-legged and the violet-legged individuals, which do not differ in any other respects.

E. noxius differs from all known African species of the genus by the practical absence of a carinula on the vertex. The only species to which *E. noxius* is more or less similar is *E. ibandana* Giglio Tos, but it differs strongly from it by the absence of the carinula of the vertex; by the longer elytron, which in *E. ibandana* does not reach the hind knee while in *E. noxius* it projects beyond it; by the lateral carinae

of pronotum, which in *E. ibandana* are obliterated in metazona; and by the colouration of the hind tibia, which in *E. ibandana* is blue basally and red apically.

E. noxius is closest to the Indian *E. alacris* (Serv.) but differs from it by the shape of the prosternal tubercle, which in *E. alacris* is slightly inflated to the apex (in *E. noxius* cylindrical); by the colouration of the hind tibia, which in *E. alacris* is light greyish-green or greyish (in *E. noxius* pink-violet or light blue); and by the lateral carinae of the pronotum, which are straight, scarcely incurved in *E. alacris* and slightly excurved in *E. noxius* (see figs. 1-4).



Figs. 1-4.—Disc of pronotum of male: (1) *Eyprepocnemis alacris*; (2) *E. noxius* sp. n. Prosternal tubercle of male: (3) *E. alacris*; (4) *E. noxius*.

According to unpublished observations by Mr. R. J. V. Joyce, this species is a serious pest of cotton and dura (*Sorghum vulgare* Pers.) in the Sudan, damaging the leaves and milky grain of the last mentioned.

***Catantops joycei*, sp. n. (Figs. 5-7).**

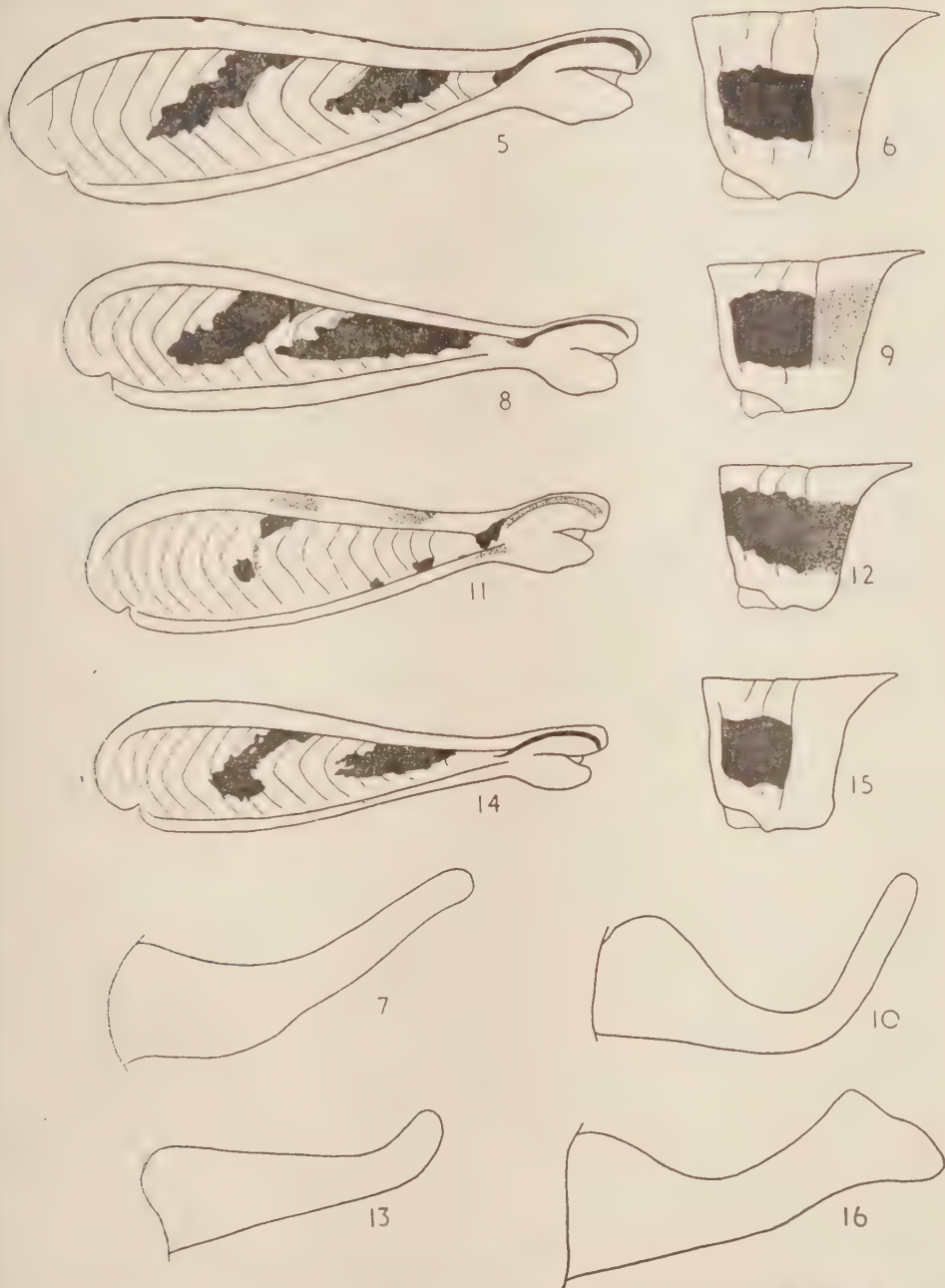
♂ (*Type*). Comparatively robust. Antennae half again as long as the head and pronotum together, slightly flattened. Frontal ridge flat, weakly widened above the ocellus and scarcely narrowed to the apex. Fastigium of vertex hexagonal, scarcely concave, half again as wide as long. Occiput with a weak carinula.

Pronotum almost flat, anterior margin of prozona truncate, posterior margin of metazona obtusangulate. Lower margin of the lateral lobe with a triangular projection in the middle. Prosternal tubercle short, cylindrical, its apex rounded, with sparse hairs. Mesosternal interspace as long as wide, with incurved lateral and anterior sides.

Elytron projecting little beyond the apex of the abdomen. Hind femur comparatively broad; lower lobe of the hind femur obtusely triangular, with curved lower margin.

Subgenital plate subconical, its upper margin in profile, almost straight, the lower one convex; apex sparsely hairy. Supra-anal plate triangular, rounded at the apex

in the basal half with a shallow median longitudinal sulcus. Cerci comparatively short, broad and robust at the base, apical half slender and moderately recurved, apex slightly compressed.



Figs. 5-16.—Hind femur, lateral lobe of pronotum and cercus of male: (5-7) *Catantops joycei*; (8-10) *C. curvicerus*; (11-13) *C. haemorrhoidalis*; (14-16) *C. melanostictus*.

Colouration yellow-ochraceous with brown pattern. Antennae yellow. Lateral lobe of the pronotum in the upper part, between first and third transversal sulci, with a wide sharp brown longitudinal stripe, which continues as a lighter stripe to the hind margin of the lobe. Elytron with numerous grey points and some small black spots along the discoidal field. Hind femur on the outside yellow, in the upper part with two oblique black incomplete fasciae, and a line along the upper lobe of the knee; above with two indistinct fasciae; inside bright red, with two small black spots opposite the external fasciae, and a small black spot in the upper part of the base. Hind tibia red, except a narrow yellow line along the external side. Hind tarsus red.

♀ (Paratype). As the male, but larger. Subgenital plate acute-triangular at the apex and with small, rounded lateral lobes.

Measurements: Length of body ♂ 23-26 (Type 23.5), ♀ 26-27; pronotum ♂ 5-5.5 (Type 5.5), ♀ 6-6.5; elytron ♂ 16-18.5 (Type 18), ♀ 19-21; hind femur ♂ 11.5-13.5 (Type 13.5), ♀ 15-15.5.

ANGLO-EGYPTIAN SUDAN: Plains 30 miles west of Gedaref, September-October 1948, 10 ♂♂ (including type), 10 ♀♀ (*R. J. V. Joyce*).

C. joycei may be confused with the following species: *C. curvicerca* Miller 1929, *C. haemorrhoidalis* Krauss 1877, and *C. melanostictus* Schaum 1853, but differs from all of them by the shape of the cercus, as well as by the pronotal and femoral pattern (see figs. 5-16).

C. joycei is a serious pest of leaves of dura (*Sorghum vulgare* Pers.), flowering heads of sunflower, leaves of cotton and leaves and young fruit of sesame (*Sesamum orientale* L.). I have much pleasure in naming this new species after Mr. R. J. V. Joyce, whose investigations on the injurious grasshoppers of the Anglo-Egyptian Sudan promise to throw light on a serious new problem in African entomology.

THE MEDITERRANEAN FRUIT FLY IN ISRAEL.

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Status in Israel.

The nature of the damage caused by the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), is well known and there is no need to redescribe it here but the amount of damage in any particular area depends upon climatic conditions and horticultural considerations. In Israel, the fruit fly has become a decisive factor since its introduction early in this century, and each variety of fruit is affected differently according to the climate of the locality in which it is planted and its proximity to other fruit trees.

In a previous paper (Rivnay, 1941) it was pointed out that the major portion of the *Citrus* season coincides with the quiescent winter period of the fly and consequently most of the crops, especially the Shamouti oranges, may escape injury. On the other hand, the early maturing varieties, such as the Clementine tangerines and grapefruit, suffer from attack in the autumn, while the late varieties, such as Valencia oranges, suffer damage from the fly in the spring. The stage of maturity of the fruit which depends upon several factors (Rivnay, 1936), determines the start of the autumn attack. The weather controls the beginning of the activity in the spring but Valencia oranges cannot remain on the tree later than 15th April without risking exposure to the fly.

The position as regards the summer fruits is worse. Apricots and peaches cannot be grown in the plains because of heavy infestation. In the hills only those fruits which ripen in the quiescent period of the fly escape, but in general these fruits must be bagged. In localities where the fly population is dense, varieties of plums, apples and pears are also heavily attacked.

The position has become still more serious in recent years as a result of the introduction of many exotic subtropical varieties of fruit. Of these the guava is most favoured, and the entire crop becomes infested unless it is picked before maturing. Mango and avocado also are favourable hosts, and some varieties must be picked before maturing, or be bagged, in order to prevent infestation.

The cultivation of fruit trees has increased a great deal lately on account of the exploitation of underground water and its distribution to neighbouring arid areas. This tendency will increase still more when the larger arid areas of the Neguev come to be irrigated with water brought from remote sources. These plans to extend fruit growing give rise to great concern.

This insect has been studied by several workers over the past 15 years, in various parts of the country and from various aspects. The present paper includes studies on the biology and ecology of the fly and factors controlling its phenology.

ECOLOGY.

Egg.

No special study was made of the length of the incubation period of the eggs on account of technical difficulties. From casual observations it is certain that, at a favourable temperature of about 25–26°C., the eggs hatch two or three days after they have been laid. At the temperature prevailing during the winter months in the maritime plain, development may be retarded to as much as 10 days and more.

Very seldom does the temperature drop sufficiently low to bring about a sudden mortality of the eggs. On the other hand, during the summer a temperature above 30°C. is undoubtedly injurious. Klein and Paker (1942) showed that, in the Jordan Valley in August, only 28 per cent. of the eggs developed into larvae, whereas 53 per cent. developed in July and 57 per cent. in October. Atmospheric humidity apparently has but little effect on eggs that have been oviposited as they are surrounded by moist tissue.

During the winter months, when they are a long time hatching, the eggs are devoured by minute mites. These enter the oviposition cavity and suck the egg, leaving an empty shell. No records are available as to the effect of these predators as, at this time, their activity is masked by other stronger factors that limit the development and activity of the fly.

Larvae.

Effects of food.

The rate of development of the larvae is influenced by food and temperature, and humidity has no influence since they are surrounded by moisture throughout their existence.

Back and Pemberton (1918) have already pointed out that the speed of development of the larvae differs with the fruit upon which they feed. In August 1933, the writer bred larvae concurrently in figs, peaches, pears and two kinds of apples at a room temperature of about 29°C. The results of these experiments are presented in fig. 1. The most rapid development took place in fresh figs, with the

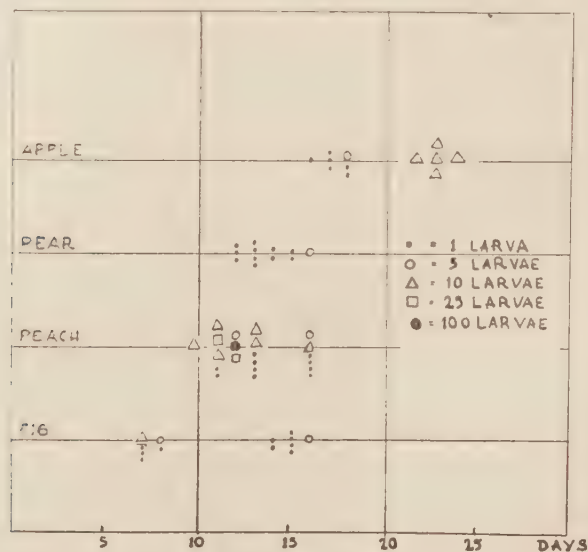


Fig. 1.—Speed of development of larvae in various fruits.

first larvae developing within a period of six days. In peaches, the development was slower and in pears still slower, while the longest development period occurred in apples. It seems that the rate of development is closely related to the physical texture of the food tissue; in apple the consistency is such that food is not easily obtained by the tender larvae. For the same reason, there is a considerable

difference in the rate of development of larvae in two varieties of apples of different texture. The composition of the food also exerts an influence and the rapid development in fig is probably due to the higher concentration of sugar therein, for according to Wehmer (1929), the fig contains 16 per cent. sugar, while the sugar content of the peach varies from 3-15 per cent. Furthermore, the percentage of water is smaller in fig than it is in peach.

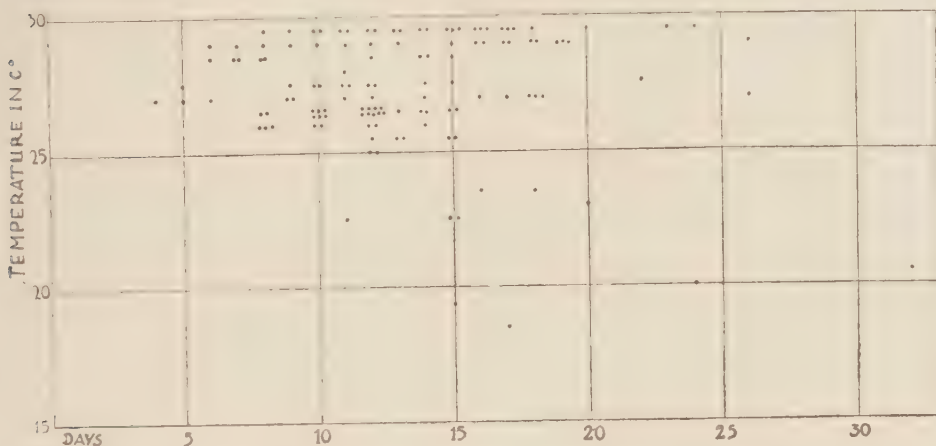


Fig. 2.—Rate of development of larvae at various degrees of temperature.

It was frequently observed that, when the medium began to ferment, the development of the larvae was retarded because, as the sugar was fermented, less food was available. This is very noticeable in the case of figs, in which some larvae remained for as much as 18 days before maturing. It is, of course, quite possible that the acidity of the fermenting juice also checked growth. In the case of *Anastrepha ludens* (Lw.), it has been pointed out (Baker & others, 1944) that the acidity of a fruit may not only retard, but also inhibit development.

Effects of temperature.

If the exact influence of temperature upon the rate of development is to be studied, the food factor should be eliminated. Care should also be taken that, when constructing the development-temperature curve, periods in which the temperature was below the threshold of development of the fly should not be included in the time and temperature calculations.

Workers often fail to observe this and it is held here that data of larvae which are lagging in their growth should not be used, for this may be due to lack of proper food. In the following calculation, the food factor was eliminated, and data were chosen from larvae bred on the same food.

The average development period was calculated for larvae reared in grapefruit at 22° and 26°C. Using these results for the calculation of the threshold of development according to the Blunck formula the following equation is obtained :—

$$15 (22-x) = 11 (26-x) ; x = 11^{\circ}\text{C.}$$

In the case of larvae reared in figs at 29° and 18.5°C. the following equation is obtained :—

$$7.5 (29-x) = 17 (18.5-x) ; x = 10.2.$$

According to the above results, the threshold of development, *i.e.*, the temperature at which development of the larvae ceases, is, therefore, between 10° and 11°C. (fig. 2).

Pupae.

Effects of moisture.

The development of the pupae depends on climatic factors only. Two tests were made with an equal number of pupae in dry and moist soil. No difference was noticed in the rate of development nor in the mortality. On the other hand 100 per cent. mortality occurred when the soil was saturated. In the citrus groves, soil conditions in the summer very rarely reach a stage where saturation lasts for many days. Most of the groves are planted on light soil but in those planted on heavy soil, during seasons of heavy rainfall, saturation of the soil may last sufficiently long to cause a heavy pupal mortality. This results in a reduction of the spring population of adult flies.

Effects of temperature.

Pupae were reared in the laboratory at various temperatures, at room temperatures and at thermostatically controlled temperatures. The results are presented in fig. 3. It will be noticed that the temperature-development curve is better defined than in the case of the larvae, since food factors play no rôle here.

If the average time-development value is calculated from the empirical values obtained in these breedings the threshold of development of pupae can be obtained, using the Blunck formula, as follows :—

$$8(29-x)=25(17-x); x=11.3$$

$$9.3(25-x)=35(15-x); x=11.4$$

$$25(17-x)=35(15-x); x=10$$

The average of these results is 11°C. which is quite near to that for the larvae. The hyperbolic curve is calculated accordingly.

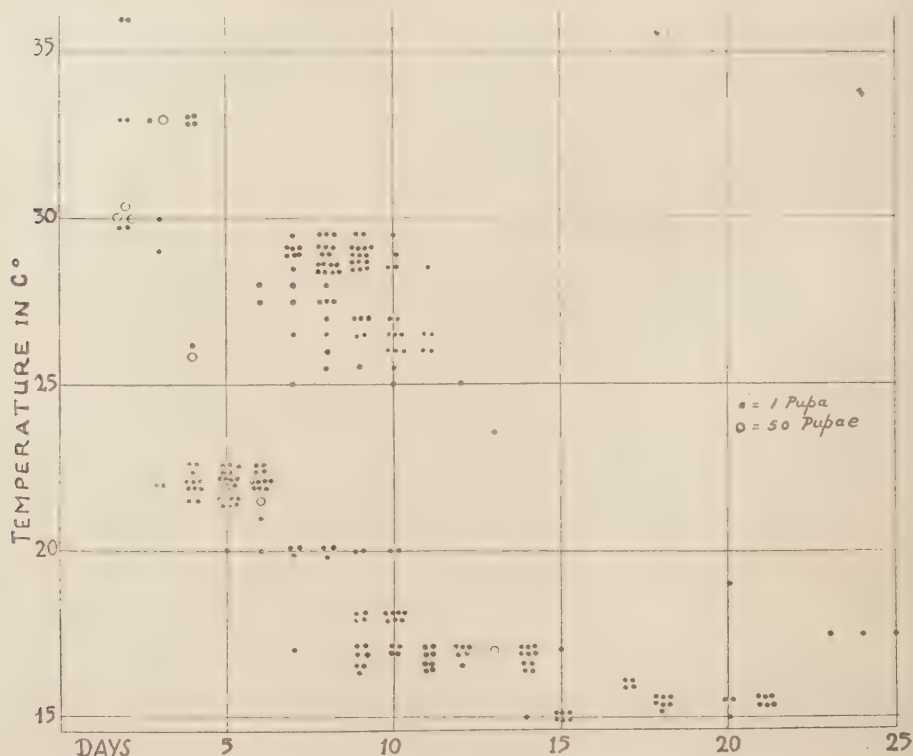


Fig. 3.—Rate of development of the pupae at various degrees of temperature.

The empiric data in the graph present what seem to be contradictory results. On the one hand the development of the pupae is retarded at the temperature of 26–29°C., creating a parabola instead of the expected hyperbola, and on the other hand at 30–37°C. the development is greatly accelerated, being completed in 2–4 days. It should be pointed out, however, that the breedings at 26–29°C. were in the room, and the flies which emerged lived normal lives and laid eggs. On the other hand those bred at 30–37°C. were maintained continuously at their respective temperatures in thermostats, and the flies which emerged died immediately or lived at most for one day. Furthermore, a great many pupae did not survive at all, which indicates that high degrees of temperature are detrimental. Further experiments were, therefore, carried out to establish the degree of mortality at higher temperatures. Pupae were placed for six, three and one hour periods each day at varying degrees of high temperature, and for the remainder of the day left at the room temperature of about 27°C. This procedure was repeated for five days. Two weeks later the emergence of flies and mortality of pupae were recorded with the following results:—

TABLE I.

Temperature	Period of daily exposure in hours	No. of pupae	No. of flies emerged	No. of flies dead
32°C.	6	25	19	6
37°C.	6	25	21	4
Control room temperature 24°C.		25	23	2
42°C.	6	25	2	23
42°C.	3	25	15	10
42°C.	3	25	17	8
Control 26°C.		25	16	9
46°C.	3	25	4	21
46°C.	3	25	6	18
46°C.	1	25	13	12
46°C.	1	25	14	11
Control 26°C.		25	17	8
50°C.	1	125	0	125
50°C.	3	125	0	125

From these figures it is evident that at 32–37°C., exposure of six hours daily for five days does not harm the pupae to any great extent, but at 42°C. such an exposure caused heavy mortality. At 46°C. one hour exposure killed about 50 per cent. of the pupae, while a 3-hour exposure killed about 80 per cent. At 50°C. all pupae died after 1-hour exposure.

Some pupae were also reared outdoors under normal conditions. Small vials containing a number of pupae imbedded in soil were buried at about a depth of 5 cm. in a grove. Some were buried at a spot on which the sun shone for most of the day, whilst others were buried in the shade of a tree. Experiments were carried out at different times of the year and pupae were reared in the laboratory to serve as controls. Survival figures are given in Table II.

The larvae usually penetrate to a depth of about 5 cm. to pupate. They do not chose a sunny or shady place for this purpose, but usually enter the soil where they drop so that many may be in the shade and many in sunny places. No attempt is made here to establish the percentage of mortality of pupae in the field under these conditions. The results in Table II, however, show that during the months of June,

July, August and also part of September, a large number of the pupae perish in the ground, because of the high temperatures in the soil in the coastal plain.

TABLE II.

Month	No. of experiments	Total no. of pupae in each case	Percentage of emerging flies		
			Shade	Sun	Indoors
April 1940... ..	1	20	85	15	70
May 1947... ..	1	25	84	32	72
June 1947... ..	1	25	52	0	48
July 1938-39... ..	4	100	71	2	74
August 1939-40... ..	2	75	46.6	4	61.3
September 1940... ..	2	75	58.6	25.3	73.3
October 1938-40... ..	3	125	83.2	85.6	79.2
November 1940... ..	1	25	96	84	80

Adult.

Preoviposition period.

The length of the preoviposition period varies greatly among individuals, even under the same conditions. At a temperature of 29°C. some flies laid 3 or 4 days after emergence, while egg-laying was retarded in others for as much as two weeks. At 17°C., some laid 15 days after emergence, but others only began to lay after two or three months. This difference in the length of the preoviposition period is due to the influence of at least four factors: (a) Food, (b) Copulation, (c) Rate of activity of the fly, (d) Rate of development of eggs.

(a) Food.—Food must be taken by the fly before it can oviposit. The composition of the food also has an influence on the speed of the development of the egg. Hanna (1947) has shown that both protein and carbohydrates must be taken up for egg-laying.

(b) Copulation.—While flies may lay infertile eggs, copulation, in addition to fertilising the eggs, probably acts as a stimulus and speeds up egg development.

(c) The activity of the fly.—In a previous paper (Rivnay, 1941), it has been pointed out that at a temperature below 16°C. a high percentage of flies remain inactive, in other words, the threshold of activity is about 5.5 degrees higher than the threshold of development. Consequently, even if a female contains ripe eggs, oviposition will not take place at a temperature below 16°C.

(d) Rate of development of the eggs. Even at a temperature above 16°C., oviposition will not take place when the eggs are not ripe as they need a definite number of time-temperature units in order to ripen. When the temperature remains below the threshold of development of the eggs for a long period, the eggs do not develop, and the preoviposition period is correspondingly prolonged.

With these details in mind, and comparing the annual temperature curve, it is obvious that egg-laying begins to decrease during November, and stops altogether in December. With the exception of extremely mild winters, egg-laying is not resumed until late in March or April, depending upon the prevailing weather conditions.

Oviposition.

The total number of eggs laid by one female in the laboratory varied exceedingly; out of 37 females, 13 laid less than 100, 16 laid between 100 and 200, 7 laid between 200 and 300 and one laid 335 eggs. The average of these figures is about 135 eggs per female. The temperature at the time fluctuated between 25 and 30°C. Flies with a low average of oviposition were bred at a temperature of 28.5–29.5°C.

The rate of oviposition is influenced by the same factors as those mentioned for the preoviposition period, but in this case the age of the individual female introduces an additional factor. If the rate of oviposition at a certain temperature is to be studied, other factors should be eliminated. In the following calculation only that period in which the females were in their optimum state was chosen—the early period and the later period of oviposition were eliminated from the calculation. At a temperature of 27–28°C., 6–12 eggs were laid each day. It seems that temperature begins to exert an adverse influence on reproduction at 29°C. It is true that at first the rate of egg-laying is much accelerated but it declines very quickly.

(3) Rate of development of ovarioles.

The effects of various temperatures upon reproduction can best be judged by observing the development of the eggs in the ovarioles. A number of flies were reared at temperatures varying from 18 to 34°C. No fruit was placed in the cage, so that no oviposition could take place. At intervals after emergence, a number of the flies were dissected and the eggs counted. Food was plentiful and uniform throughout the period, and consequently all the factors were uniform with the exception of temperature. Seventy-five flies were bred at a temperature varying from 18–22°C.; 207 at 23–25°C.; 54 at 27–29°C. and 41 at 33–34°C.

These flies were dissected at given intervals, and the number of mature eggs counted. Fig. 4 shows the number of eggs that matured during given intervals at the various temperatures. It is obvious that the development of eggs is greatly retarded at a temperature of 18–22°C. (curve 1); after one month only 3–5 eggs were found in the ovaries. At a temperature of 23–25°C. (curve 2) the development of eggs is slightly retarded, but progresses continuously, so that at the end of a month over forty eggs are found in the female.

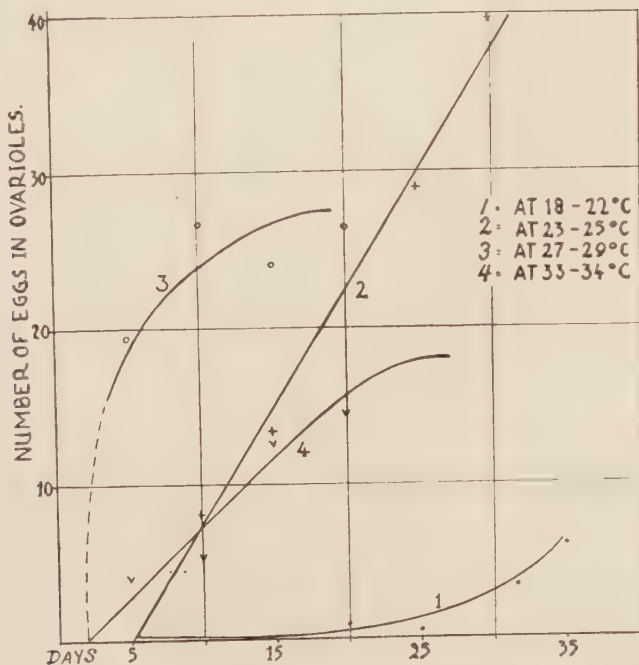


Fig. 4.—Development of eggs in the ovarioles at various degrees of temperature.

The development of eggs is greatly accelerated at a temperature of 27–29°C. (curve 3), but, when flies are exposed to it for a longer period, the progress of development is unlike that at 23–25°C. and a marked drop takes place, indicating that the higher temperature is exerting an adverse influence on reproduction. It is quite obvious that at 33–34°C. the reproduction is abnormal (curve 4) for although the development after five days is higher than at 23–25°C., in the end it lags far behind. In addition, the fly is very shortlived.

From this table it is apparent that the temperature most favourable for reproduction lies between 25–27°C.

Length of life.

In all breeding experiments carried out at room temperature the length of life of each fly was carefully recorded. In addition, flies were reared in a thermostat at higher temperatures. Thirty were bred at room temperature between 13 and 15.5°C. ; 100 between 16–19.5°C. ; 40 between 20–23.5°C. ; 300 between 24–30.5°C. and 50 in the thermostat at 37.5–38°C. so that altogether the life records of 520 flies were obtained.

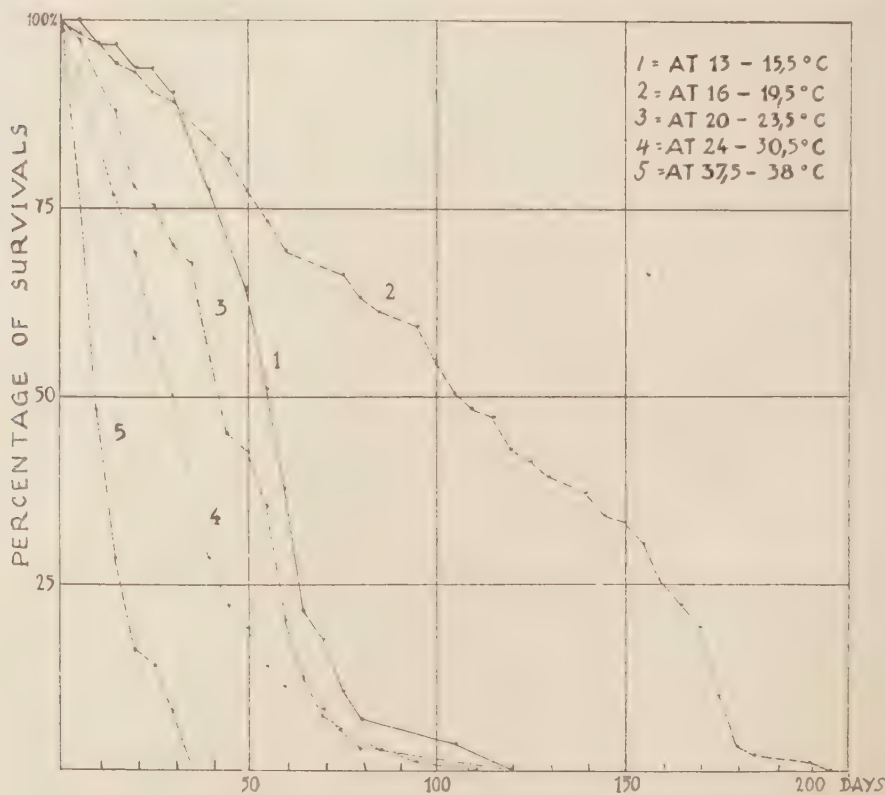


Fig. 5.—Length of life of adult flies at various degrees of temperature.

The percentage of survivals at different temperatures after varying periods of time is shown in fig. 5. At 37.5–38°C. (5) over 50 per cent. died after ten days and all were dead in just over a month. Survival was longest at 16–19.5°C. (2) when 100 days elapsed before 50 per cent. mortality was reached and about 200 before all were dead. When the temperature fell below 15°C. (1) mortality was accelerated and 50 per cent.

were dead after 55 days. At the optimum range of 24–30.5°C. (4) 50 per cent. of the flies died after 30 days, but nearly four months elapsed before all were dead.

Bearing in mind the conditions prevailing during the winter months, it is concluded that, even without host fruits, females may easily survive the winter.

PHENOLOGY.

The Observation Grove.

In order to study the fluctuation of the fly population and the factors controlling it in the coastal plain, a grove of subtropical fruit trees, providing a food supply throughout the year, was chosen for observation. This grove, about 8 dunams (2 acres) in extent, was surrounded by citrus groves on all sides. On the south and north they were adjacent, while on the east and west the groves were at a distance of about 100 and 500 metres, respectively. In addition, there were some citrus trees in the observation grove ensuring continuity of food supplies for the entire year.

Early grapefruit in September and October, Shamouti oranges up to April and Valencia oranges in April and May provided a succession of suitable fruits throughout the winter. In May there were also Pitanga (*Eugenia pitanga*) and Neffles (*Eriobotrya japonica*) available, but long experience has showed that Neffles are never attacked, while Pitanga are only infested on certain occasions. Apparently these two fruits cannot compete with ripe citrus, which may still be on the trees. In June, especially late in the month, *Eugenia jambos* was severely infested. Other species of *Eugenia* and *Psidium guajava* followed in July and August and in addition many species of mango and avocado became sufficiently ripe to be attacked by the fly by August and September. During the latter month, oriental persimmon (*Diospyros kaki*) and *Annona* were added to the list of host fruits thus completing the chain of hosts throughout the summer carrying and ensuring a fly population to infest the new Clementines and grapefruit in September and October.

Trapping.

Trap jars hung on various kinds of fruit trees in all sections of the grove were used. The jars were of the usual design with the opening beneath. The bait liquid consisted of a 5 per cent. solution of "Clensel", and was changed every five days. Counts were made of males and females, and ten-day records compiled. It is assumed that an increase of the fly population in a certain area is reflected in an increase of the number of flies trapped and that, although the number trapped represents only a certain percentage of the population, the catches will give a fair representation of the changes in population as a whole.

For the first five years, about 30 jars were hung in the grove, five in each row of a certain kind of fruit tree. Later, when the number of flies increased, the number of jars was reduced to three jars in each row. The number of flies in the figures presents the average per trap jar in each case.

The trees upon which the jars were hung were, first row in the east—pomegranates; the second row, 12 metres away—pitanga; third row—guava; fourth row—mango; fifth row—*Annona*; sixth row—citrus.

The percentage of flies caught on each type of tree varied with the season. The blossoming and fruit-bearing season of certain trees usually attracted many of the flies to that particular row of trees. This was not always the case, however, for certain jars always had lower catches than others. The ecological surroundings of the jar had a considerable influence on the catch; in the summer, a jar in the shade took more flies than a jar exposed to the sun.

Figs. 6-9 in general are based upon the average number of flies per jar. There is no reason to ascribe the larger number of flies in latter years to the smaller number of trap jars in the grove. It is evident that in spite of the smaller number of jars, the total number of flies trapped increased and the writer is convinced that the averages per jar are a true representation of the fluctuation of the fly population.

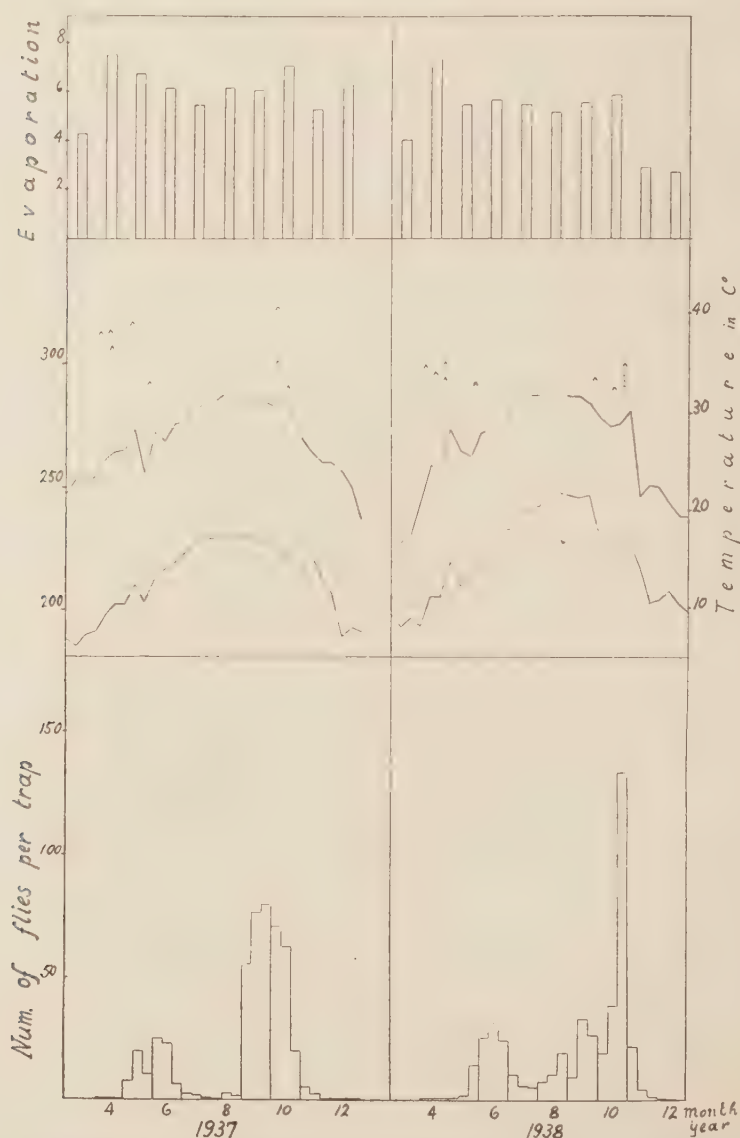


Fig. 6.—Fluctuations of the population of the fly at Rehovot during 1937 and 1938. In figs. 6-9 the upper section represents the monthly average of evaporation in mm. per day. The middle section represents the average of the maximum and minimum temperatures for every ten days. The wedges indicate the absolute maximum temperature on days when it rose to beyond 30°C.

Influence of Availability of Fruit on the Increase of Flies.

The presence of the fly in a certain area depends upon the availability of suitable fruits. An abundance of fruit leads to an increase in the population, provided the climatic factors are favourable. The influence of this factor can be seen from the graphs illustrating the fluctuations of the fly population in the observation grove during the years 1937-44 (figs. 6-9 inclusive).

In 1937 and 1938 (fig. 6) the flies were least numerous because the trees were young and bore little fruit, many in fact did not bear at all. But, as they grew older, more trees of all varieties came into bearing and the quantity of fruit produced

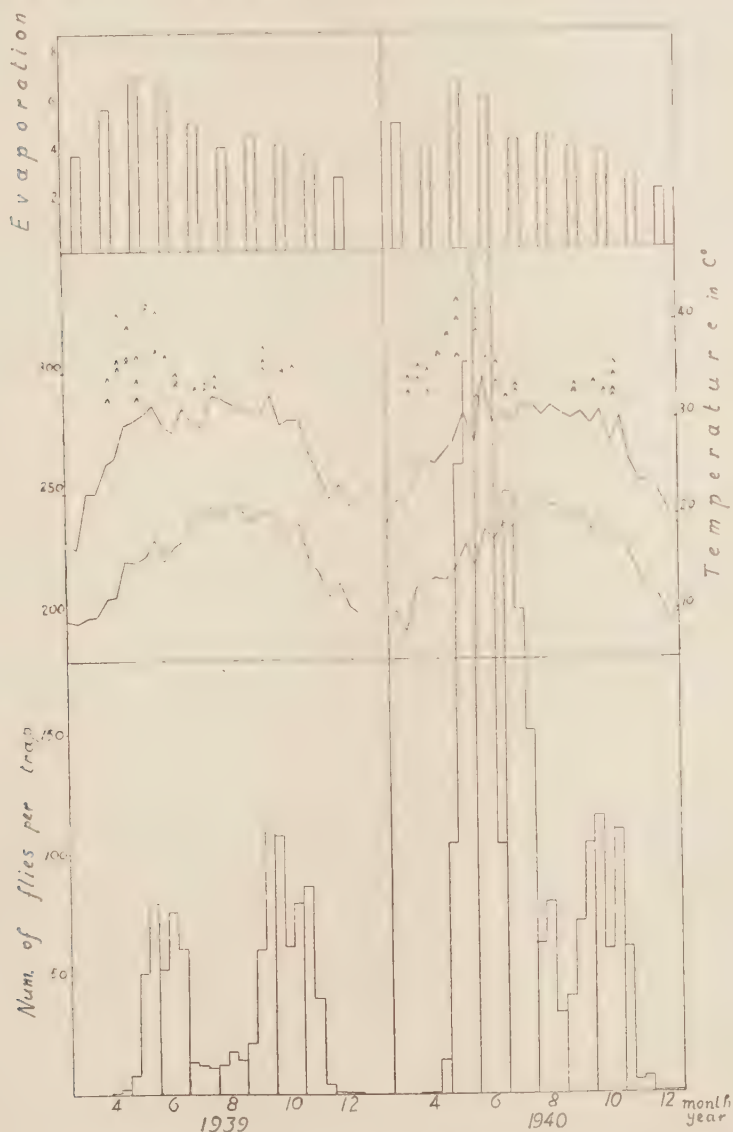


Fig. 7.—Fluctuations of the population of the fly at Rehovot during 1939 and 1940.

increased. This accounts for the difference in the fly population between these years and that of 1943 and 1944.

In 1940-41 (figs. 7-8) the spring population was out of all proportion. On many occasions the number of flies per jar amounted to over 4,000. The explanation for this lies in the fact that, for the first two years of the war, the citrus fruit was neither marketed, nor used for the manufacture of by-products. It was allowed to remain on the trees until May and June, thus giving rise to an excessive fly population. From the adjacent citrus groves dense swarms of flies migrated into the observation grove and swelled the population out of all proportion. These migrations continued throughout the months of May and June. During the latter years of the war, the citrus industry adjusted itself to war conditions. On the one hand, lack of cultivation resulted in a decrease of fruit in the citrus groves and on the other hand, the fruit was picked early, so that by May there was none available. Consequently, the number of flies caught in 1943-44, although influenced by the adjacent citrus groves, presents a normal picture of the population.

Parasitism.

In November 1947, an *Opius*, the specific identity of which has not yet been established, was reared from infested guavas but the percentage parasitism was so small that it can hardly be considered as exercising any influence upon the fluctuation in the number of flies. This wasp can attack only larvae located close to the surface. Most of the fruits subject to infestation have a thick skin and are rich in pulp, and the parasite cannot reach the larvae, which are deeply embedded. During the years 1937-44 no parasites were found at all.

The Fly Population Month by Month.

December-March.

In a previous paper (Rivnay, 1941) it was pointed out that the weather from December to March in the coastal plain is too rigorous for the fly. Development at the temperature prevailing is greatly retarded, while oviposition is negligible despite the abundance of fruit. Hardly any flies were trapped during this period. The fly on the wing appears in the spring when the average temperature rises to about 17-18°C., the date of appearance depending upon the weather conditions during March. The subsequent rate of increase of the population is determined by the weather during April and May.

April.

The fly population in April is composed of over-wintering adults and new flies that have emerged from overwintering pupae. If March is mild there may be a third source arising from a new generation from eggs laid in citrus fruits by overwintering females early in that month and these may be on the wing late in April. A prolonged rainy season with cool weather late in the spring retards the appearance of the flies. On the other hand, a mild February and March favours the early presence of flies on the wing. There were no flies in April 1937-42-43 (figs. 6, 8, 9) because of the low temperatures that prevailed in the months of March and April of these years. On the other hand in April 1944 (fig. 9) flies on the wing were abundant.

May and June.

From the end of April and onwards the fly population in the observation grove increased very rapidly. The amount and rate of increase of the population then depended upon the dryness of the spring.

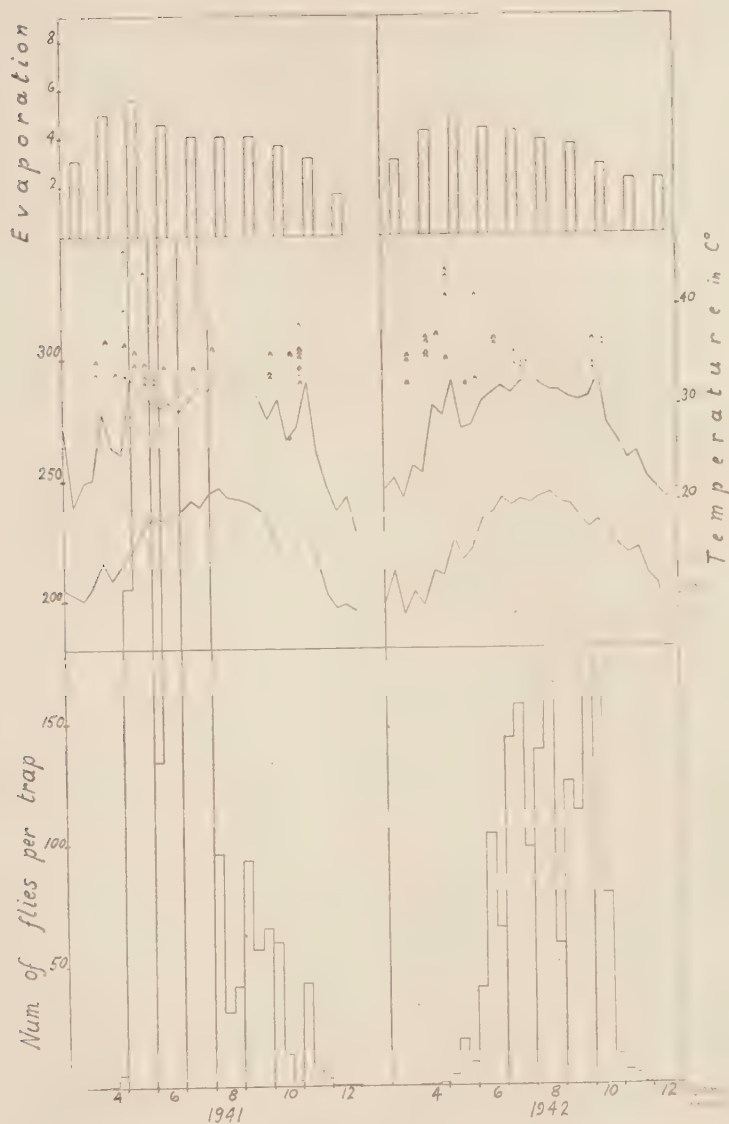


Fig. 8.—Fluctuations of the population of the fly at Rehovot during 1941 and 1942.

As a rule, during this period severe and dry hot desert winds prevail and their ill-effects upon the flora and fauna of the country is conspicuous. On the occasions when these winds are severe, *e.g.*, when the temperature is very high and humidity very low and of long duration, the fly population diminishes greatly. But when the weather happens to be mild and warm, and not dry, the fly population swells. The low population of May and June 1937, 1938 and 1939 should be compared with the population for the same months of 1943 and 1944, and the difference in temperature, evaporation and khamsin days noted.

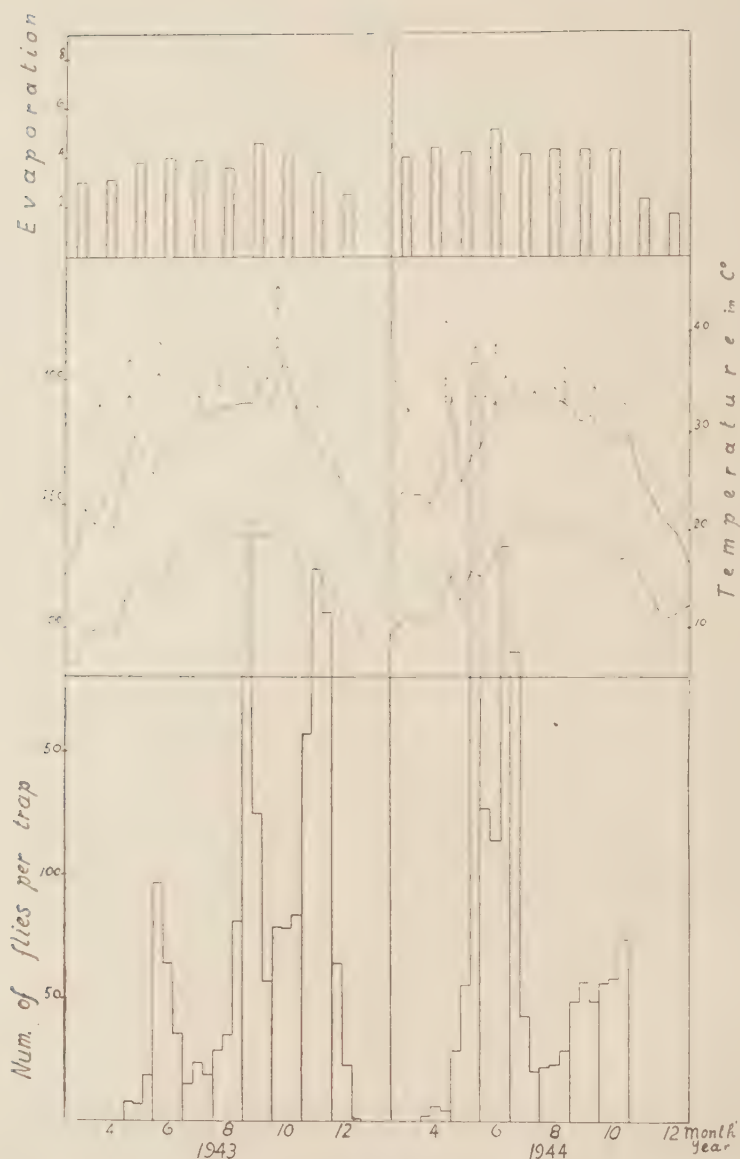


Fig. 9.—Fluctuations of the population of the fly at Rehovot during 1943 and 1944.

July and August.

Comparing the fluctuations in the fly population for the eight years it will be noted that, with the exception of 1942, there is a distinct drop in July and August. The absolute number of flies during these months depends upon the number in the previous May and June. Compare for instance the curve for July and August 1937, 1938 and 1939 with that of the respective months of 1943 and 1944. The depression may be due to two reasons.

Firstly, the lack of available host fruit should be considered. The flies on the wing in April and May certainly originated from overwintering females and pupae and a great many migrated to the observation plot from the surrounding citrus groves. It has already been pointed out that there is no fruit suitable for oviposition except citrus during May and June. The neffles, *Eriobotrya japonica*, which mature in May remain free from attack and the pitangas which ripen in May and June are likewise free from infestation. The result is that many flies die leaving no offspring and the population dwindles. Suitable fruit for oviposition appears only late in July, when the various species and varieties of *Eugenia* and *Psidium* ripen. With the approach of August and September many other varieties, such as mango and sapotas, become available.

Secondly the weather is an important factor. The continuous high temperature coupled with low relative humidity exerts its influence upon the adult. It has been shown earlier that a continuous temperature above 33°C., regardless of the humidity, shortens the life of the fly and also reduces fertility. This factor, when coupled with a relative humidity below 30 per cent. (Rivnay, 1941) accentuates the adverse influence. During July and August, the temperature often rises beyond 33°C. and even above 37°C. These temperatures may only last a few hours, but when repeated daily they have a marked effect.

It has already been pointed out that at a temperature above 42°C. the mortality of pupae increases even when exposed for six hours, while at 46°C. even one hour exposure is detrimental, and 50°C. brings immediate death. The temperature of the soil during the summer months in the coastal plain rises as high as the degrees mentioned above.

The mortality of pupae in the shade has been shown to be over 50 per cent., while in the sun less than 5 per cent. survived in July and August, and only 25 per cent. in September. Many larvae pupate in the shade, but many probably pupate in a place exposed to the sun for some hours each day. There is no doubt that the mortality of pupae is also an important factor in thinning out the population in July and August.

September and October.

During September and October there is a rise in the population. On the one hand the host abundance is an important factor, and on the other the climate is milder. The temperature is lower, the soil is cooler and the mortality of flies is much lower than in the previous month. As a rule, the peak of the population during the autumn is higher than in the spring; this was the case in all years with the exception of 1940 and 1941, when the population was augmented by the influx of flies migrating from abundant citrus fruit in the spring.

In the autumn, however, the rise and fall of the population does not run smoothly. The number of flies diminishes in accordance with the changes of temperature and humidity. In 1943, at the end of September and in October, the dry hot khamsin days brought about a reduction of the population. November, however, was mild and the rains came late, so that the number of flies on the wing increased again, and diminished only when cold weather set in in December.

November and December.

Generally speaking November and December are the months when the number of flies on the wing declines until they disappear altogether. The rate of reduction again depends upon the weather only. Early rains and low temperature bring a sudden reduction but should the winter be mild and warm, with low rainfall, the fly may be found throughout the winter, in small numbers.

The Situation in 1942.

The foregoing discussion explains the picture presented by the figures for all the years except 1942. During this year the population in the observation grove was not regular. The flies on the wing appeared quite late and disappeared very early, but they persisted throughout the summer, showing no signs of the usual depression during July and August. If the average temperatures for May and June of that year are compared with those of other years, they will be found to be much lower. June in particular remained very cool. Consequently migration from the citrus grove was delayed for about 40 days and this is evident from the low population during May and early June as compared with other years. Abandoned citrus fruit in the adjacent groves was still abundant, and, had it not been for the cool weather of the spring and early summer, the situation would have been as in 1940 and 1941, and the volume of flies would have swelled as in April and May of those years. The migration of flies was retarded, but continued throughout the latter part of June, July and early August but, in the meantime, limiting factors began to act adversely upon the flies in July and August, and prevented the volume of flies from reaching the high peaks which they reached in May and June of 1940 and 1941. The population remained within the limits shown in the graph (fig. 8).

An interesting feature for this year (1942) is the distinction of the peaks representing the generations which developed during the spring and summer of that year. The cold weather in October brought the fly population down suddenly.

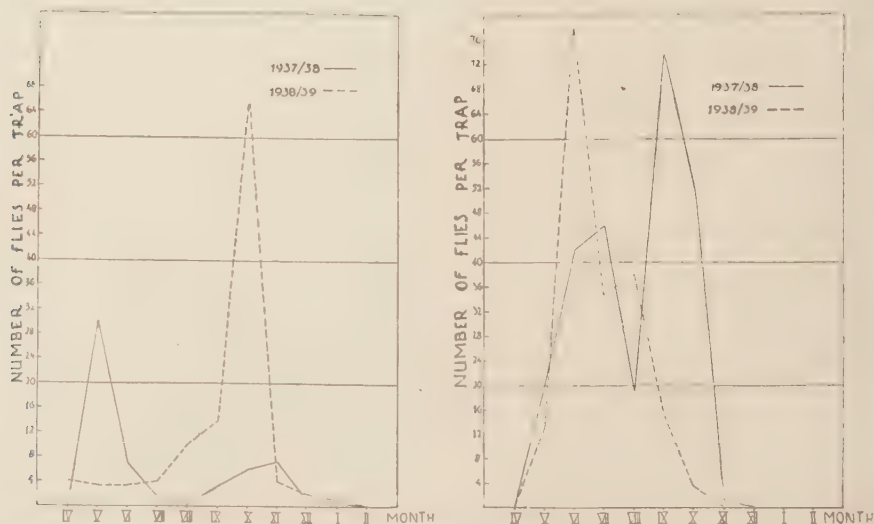


Fig. 10.—Fluctuations of the population of the fly at Mikveh-Israel. Left—Citrus grove. Right—Deciduous fruit tree orchard.

PHENOLOGY IN OTHER LOCALITIES.

The phenology of the fly in the observation grove at Rehovot is very similar to that in other places in the maritime plain. Parallel trapping of flies was carried out by H. Z. Klein (1945) at Mikveh-Israel about 15 km. north of Rehovot and at Hedera, 45 km. still further north. The same method of trapping was used except that the Clensel was changed weekly and the number of flies was recorded every week.

At Mikveh-Israel it was carried out for two years, in 1937 and 1938 both in a citrus grove and in an adjacent summer fruit grove. The latter contained deciduous as well as subtropical fruit. At Hedera the trapping was carried out for three years 1937, 1938 and 1939 in two citrus groves, around one of which an *Aberia caffra* hedge was planted.

The fluctuation of the fly population is shown in figs. 10 and 11 and the figures are based upon the average number per jar.

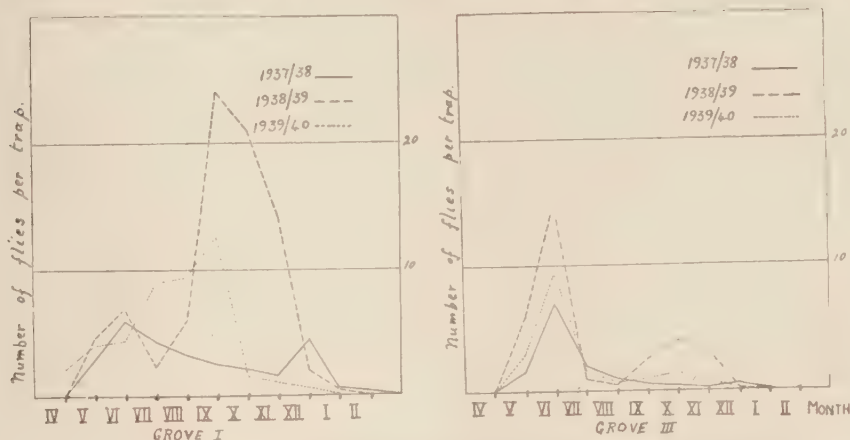


Fig. 11.—Fluctuations of the population of the fly at Hedera (after Klein).

In each of the ten records, with the exception of that for 1939 at Hedera, there are two peaks the one in May and June and the other from the latter end of August to early October with a summer depression in July–August and a winter depression from October to April. The weather determines the dates of the rise and fall in the number of flies, as well as the relative numbers from year to year. Hosts also play a noteworthy rôle. There was no summer depression in July–August of 1939 (fig. 11). This, as Klein explains, was due to the fact that, although the weather conditions of that particular summer were favourable to the flies, the fruit of *Aberia* was allowed to remain on the hedge until September, whereas in 1937 and 1938, it was removed early in the summer and buried.

The difference in the proportion of the number of flies in the citrus grove and summer fruit grove each year at Mikveh-Israel is worthy of note (fig. 10).

Systematic trapping was carried out by the writer in the Gaza district during the summer of 1943. Six trap jars were hung on peach and apricot trees; pear, plum and apples hardly attracted any flies. The first fly was caught on 17th May, and 19 were caught before the end of May. In the first half of June, 158 flies were caught, in the latter half 135, and in early July 35 were trapped, but after this no further flies were taken. This corresponds with what is found in the coastal plain and the absence of a second peak was due to the lack of late summer host fruit.

To summarise the fluctuation of the fly population in the coastal plain, the earliest flies on the wing usually appear in late April, they increase to large numbers in May–June, decrease again in July–August, and rise in September–October, after which they decrease in November and disappear entirely towards the end of December.

Fluctuation of the Number of Flies in the Upper Jordan Valley.

The comparative influence upon the fly population of the weather and the availability of host is well illustrated in the Jordan Valley. A thorough study of the flies

in this area was made by H. Z. Klein and Paker in 1938, 1939 and 1940. According to these authors, fruits are plentiful throughout the year; citrus fruit is abundant for practically nine months, and in the summer, in addition to figs and other sub-tropical fruit, grapes and tomatoes are subject to attack. Previous to this a similar study in that locality was carried out by A. Grünberg in 1935-36 (1948), who also stated that during the summer figs, grapes and cactus fruit were plentiful and subject to attack. The data given by these writers indicate that the summer depression is more pronounced than in the maritime plain. Fig. 12 gives the data obtained by Klein. Two peaks it will be noticed are present in each year, but the summer depression in each case is strongly marked. The spring peaks are always very much higher than the autumn peaks; this is apparently due to the weather conditions during the summer period being so much more severe than they are in the coastal plain. The setback to the fly population is so great that it can only rise again to its normal level after the onset of the mild and favourable weather of the winter.

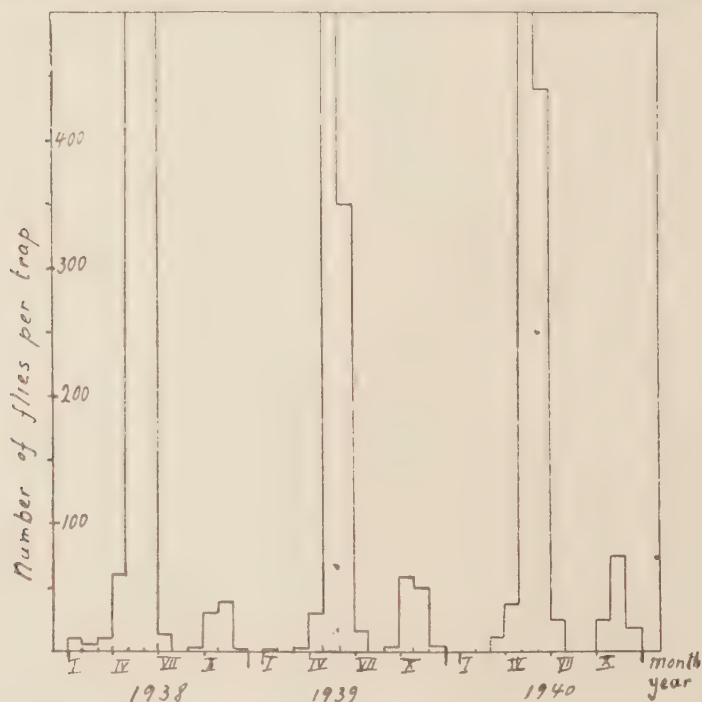


Fig. 12.—Fluctuations of the population of the fly in the Jordan Valley (after Klein & Paker).

In the Hills.

A study of the phenology in the hills was made by the writer at Kfar Giladi, Ein Hashofet, Maaleh Hakhamisha and Ramat Rachel. In these places continuous study was not possible and fig. 13 is based partly upon trapping and partly upon observations.

In Kfar Giladi, in the upper Galilee, about 300 metres above sea level, six traps were hung in April 1937. The bait was examined and changed every three days, but no flies were trapped until 22nd June. Altogether, the six traps caught three flies in June and about 30 in July. On a second occasion, in 1943, the first fly was caught on 16th June. During that month 18 flies were trapped and in July 70. The increase

in the density of the fly population in 1943 was apparently attributable to the increase in fruit production. At Ein Hashofet, on Mount Carmel, about 160 metres above sea level, six jars were hung late in May 1943. The bait was examined and changed every five days. The first fly was caught on 12th June. During that month three flies were caught altogether, while 156 were taken in July.

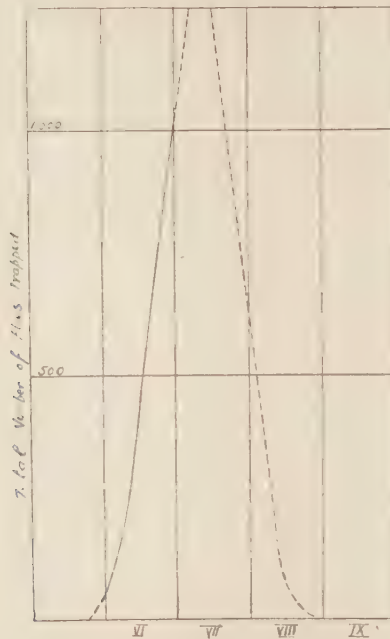


Fig. 13.—Fluctuations of the population of the fly in the Hills of Israel.

At Maaleh Hakhamisha, west of Jerusalem, about 700 metres above sea level, six traps were hung late in May 1944. The first fly was caught on 14th June. In 1946 the traps were hung early in June, and the first fly was trapped on 11th June. In the two days from 11th to the 13th of that month, 162 males and 270 females were the caught in six jars; apparently due to a dense swarm of flies being attracted to some ripening peaches. In July of that year several hundreds were caught in each trap.

At Ramat Rachel, south of Jerusalem, about 700 metres above sea level, six traps were hung in May 1943. The first fly was caught on the first of July. Altogether seven flies were caught in July and two in August. Nothing was trapped during September and October.

A study of these records suggests that the appearance of the fly on the wing in the hills is retarded by five or six weeks according to the weather. As a rule, flies in larger numbers are present towards the end of June and early July. In view of the fact that peaches, apricots, figs, etc. are plentiful then, the population during July increases extremely rapidly to many times that of June. In July and August the weather is milder than in the plain, and the high level of density of the population is maintained until the middle of August. September in the hills is much too cool for the fly and also by then most suitable fruit has been picked, so that the population drops quickly, much more quickly than in the plain, and remains at a low level until June of the following year. The main difference between the phenology of the

fly in the hills and in the plain is that in the former there is a rather short seasonal appearance of a dense population with only one peak during the year, in July, and no flies on the wing for nine months of the year, from late September to early June.

Summary.

The status of the Mediterranean fruit fly in Israel is reviewed briefly. Apricots, peaches and guavas are the most severely attacked of the summer fruits.

The ecology of the various stages of the insect is discussed. It is pointed out that, in addition to temperature, the type of food influences the length of development of the larva. The threshold of development in the temperature development hyperbola has been calculated and found to be between 10–11°C.

The threshold of development of the pupae was found to be close to 11°C. A daily exposure of the pupae for six hours at a temperature of 32–37°C. caused no injury, but a similar exposure at 42°C. brought about a heavy mortality. At 46°C. one hour's exposure resulted in considerable injury whilst three hours' exposure killed 80 per cent. of the pupae. This explains the very high mortality of the pupae in the groves during June, July and August.

A study of the development of the eggs in the ovarioles at various degrees of temperature indicated that at 18–22°C. no appreciable development took place and at 23–25°C. it was still retarded. The optimum development temperature was found to be 27–29°C., whilst 33–34°C. was actually detrimental.

A study of the length of life of the adult showed that survival was longest between 16–19°C. at which temperature it took 100 days to reach 50 per cent. mortality and 200 days a 100 per cent. mortality.

The fluctuation of the fly population in Israel is discussed in detail. It is pointed out that the prevalence of host fruits is one of the great factors responsible for these fluctuations. An analysis of the climatic conditions month by month and its influence upon the fly population is discussed. The curve depicting the fluctuations of the fly in the coastal plain has two depressions, one between December and January, and the other in July and August. In the Upper Jordan Valley, the summer depression was more clear-cut than in the maritime plain, and the spring population reached a much greater height than that in the autumn.

The fly appeared about two months later in the hills than in the plain, namely towards early June, and it disappeared also earlier, presenting a single peak in July and August.

References.

- BACK, E. A. & PEMBERTON, C. E. (1918). The Mediterranean Fruit Fly.—Bull. U.S. Dep. Agric., No. 640, 43 pp.
- BAKER, A. C., STONE, W. E., PLUMMER, C. C. & MCPHAIL, M. (1944). A review of studies on the Mexican Fruitfly and related Mexican species.—Misc. Publ. U.S. Dep. Agric., no. 531, 155 pp.
- GRÜNBERG, A. (1938). The Mediterranean Fruit-fly (*Ceratitis capitata*, Wied.) in the Jordan Valley.—Bull. ent. Res., **29**, pp. 63–76.
- HANNA, A. D. (1947). Studies on the Mediterranean Fruit-fly, *Ceratitis capitata* Wied. II. Biology and control.—Bull. Soc. Fouad Ier Ent., **31**, pp. 251–285.

- KLEIN, H. Z. (1945). The Mediterranean Fruit-fly and its Status in the coastal plain of Israel. [*In Hebrew.*]—Reshumoth Haclaioth (Agricultural Notes) Rehovot, **1**, pp. 85-95.
- KLEIN, H. Z. & PARKER, M. (1942). Studies on the biology of the Mediterranean Fruit Fly in the Jordan Valley. [*In Hebrew with English Summary.*]—Bull. agric. Res. Sta. Rehovot, no. **32**, 33 pp.
- RIVNAY, E. (1936). Infestation of oranges by the Mediterranean Fruit Fly during the autumn in Palestine.—Hadar, Tel-Aviv, **9**, pp. 134-137.
- RIVNAY, E. (1941). The activity of the Mediterranean Fruit Fly at cold temperatures with reference to its status, during the Citrus season in Palestine.—J. ent. Soc. sthn Afr., **4**, pp. 166-176.
- WEHMER, C. (1929). Die Pflanzenstoffe. Jena.

STUDIES OF BRITISH ANTHOMYIID FLIES.

By Mary MILES.

*Wye College, University of London.*I. BIOLOGY AND HABITS OF THE BEAN SEED FLIES, *Chortophila cilicrura* (ROND.)
AND *C. trichodactyla* (ROND.).

Preliminary observations on the occurrence of the bean seed flies, *Chortophila cilicrura* (Rond.) and *C. trichodactyla* (Rond.) have already been published (Miles, 1948) and more detailed observations have now been made with the aid of a grant from the Agricultural Research Council. As in the previous paper, the name *C. cilicrura* is used to cover both species until examination of bred material is completed.

Adults.

Hawley (1922) observed that the flies were attracted to moist, newly ploughed soil in the spring. Observations at Wye in 1948 showed that the flies came to freshly turned soil at all times between April and October. Any disturbance of the soil by ploughing, digging, hoeing, weeding, raking, or setting out of plants served to attract them. The bare soil of experimental plots has been watched and no bean seed flies seen but, within a few minutes of raking or hoeing, the flies could be found on the newly broken surface. They continued to be active on fresh soil for some hours, but the greatest numbers were found in the first two to three hours after it had been disturbed. So constant was the attraction of fresh soil to the flies that it could be used throughout the season to collect specimens for breeding. Other Diptera came to the soil, but they appeared to be occasional visitors and did not explore the soil crevices in the manner of the bean seed flies.

The number that came to the freshly turned soil of the observation plots was affected by cultural operations in progress in the immediate neighbourhood. The observation plots were situated on a three-acre field used for intensive vegetable production and on a 14-acre field used for extensive vegetable growing. When no cultural operations were in progress on the surrounding field, any disturbance of the soil on the plots (3 yds. \times 3 yds.) gave rise to a concentration of 0-4 flies per square foot. It was not possible to assemble flies on the plots when ploughing, hoeing, or irrigation was in progress on the surrounding field but examination showed that they were active and widely dispersed at concentrations of 0-3 per square yard over the whole of the disturbed or irrigated area.

The weather also affected the activity of the flies. They assembled most readily on the observation plots in dry warm sunny weather; in damp weather they were difficult to attract, probably because of the widely dispersed odour of damp soil. On dull cold windy days the flies came to freshly disturbed soil and could be found crawling among the crevices and soil aggregates, but they were more difficult to see when there was no sunshine to light up their iridescent wings. The flies were very sensitive to shade and movement on bright days and took to flight at once when disturbed. In cool weather and intermittent sunshine, they sought shelter under small clods and in crevices, disappearing from the surface when it was dull and reappearing when the sun shone again.

Hawley (1922) found that females came more readily to the soil than males and this appeared to be the case during the present observations. Of 162 flies taken on

the soil at various times during the season, only 33 or approximately 20 per cent. were males. Since the flies were caught individually, it is possible that the lighter colour of the females made them more conspicuous on the soil than the males and thus introduced bias into what appeared to be a random selection. The longer life of the females (p. 346) may also have affected the proportions of the sexes.

Mating appeared to take place on the wing in sunshine. Attempts at mating were seen when captive flies were exposed to sunshine. Pairs of flies were sometimes seen to fall to the ground over freshly turned soil.

The attraction of the soil seemed to have some association with egg-laying since flies taken on the soil sometimes laid eggs within ten minutes of their capture. The flies were also observed to shelter for short periods in the soil and it appears that longer periods of inclement weather may be passed in the soil since Rekach (1932) observed that in Azerbaijan the flies hibernated in the ground or under stones and clods.

Males in captivity lived up to 24 hours and females up to 48 hours when neither food nor moisture was available, but when provided with damp soil or damp filter paper they lived up to seven days, females usually persisting longer than males. When filter paper moistened with 5 per cent. sugar solution was placed in the phials, the flies were stimulated to activity and within a few moments found the filter paper and began to feed. The life of the flies was greatly extended and females laid eggs in captivity when sugar solution was continuously available.

Observations on feeding habits under natural conditions have not yet been made, but the flies have been captured on the flowers of stitchwort, charlock and cauliflower.

Oviposition.—Other workers (Harukawa & others, 1930, 1933; Rekach, 1932) have recorded that the period between emergence and egg-laying in *C. cilicura* varied from 7 to 100 days according to the temperature and nutrition. Observations in 1948 showed that a considerable time might elapse between fertilisation and egg-laying but, as they were made on captured flies, the observed pre-oviposition period was necessarily incomplete and may have been affected by the nature of available food.

Most females laid eggs within 2–3 days of their capture but the following Table shows the length of time between capture and egg-laying in some females captured between mid-September and mid-October.

No. of flies	No. of days in captivity before egg-laying
6	7
4	12–14
2	17–18
1	29
2	35
1	38
1	44

The full egg-laying capacity has not been observed because it has not been possible to provide bred flies with suitable conditions for mating. Reid (1940) has recorded that one female laid 150 eggs and that four females laid an average of 97 eggs each. The number of eggs laid by captured flies in the course of this investigation depended on their age at the time of capture, their nutrition and survival in captivity. Many laid less than 20 eggs but probably they had already laid eggs before they were captured. The highest number of eggs recorded was 53. The following Table gives some indication of the numbers produced by flies captured in early autumn. Records of less than 20 eggs are not included.

10 flies laid	20–29 eggs each
2	"	"	...	30–39 " "
2	"	"	...	40–49 " "
4	"	"	...	50–53 " "

Captured flies were examined daily and eggs were removed from the phials. The numbers of eggs per day varied from 0 to 42 with low numbers predominating. The following data show the size of the daily egg batches.

24 records of	1 egg in the course of 24 hours
37 " " " "	...	2-5 eggs " " " " 24 "
22 " " " "	...	6-10 " " " " 24 "
17 " " " "	...	11-20 " " " " 24 "
7 " " " "	...	over 20 " " " " 24 "

The duration of the egg-laying period was found by Reid (1940) to vary up to 20 days. At Wye, captured flies laid eggs over periods up to 28 days. The following Table gives the duration of the egg-laying period of some captured flies. Periods of less than ten days have been omitted.

No. of flies	No. of days between first and last eggs
2	12-14
2	17-19
6	21-28

The duration of the egg-laying period is probably an important factor affecting the numbers of flies on the wing. Flies could be found on the wing at all times from April to October in 1948, and differences in their behaviour in captivity were probably the result of differences in age at the time of capture. In captivity, it was not unusual to have females alive and active when the progeny emerged, and this overlapping of generations may occur out of doors.

The observations on captured flies maintained in artificial conditions gave little indication that the species had a definite pattern of egg-laying. Although a number of flies were kept under similar conditions of temperature, light and food supply, egg production was irregular. The following are the oviposition histories of four flies captured in early autumn.

Fly A		Fly B		Fly C		Fly D	
No. of eggs	Days after capture	No. of eggs	Days after capture	No. of eggs	Days after capture	No. of eggs	Days after capture
1	1	1	7	1	1	11	29
1	8	7	14	4	10	3	30
11	9	1	21	4	24	1	37
16	11	1	25	11	26	2	38
		12	33	25	27	4	39
						11	41
						7	44
						9	45
						3	46

Other observers (Harukawa & others, 1930; Rekach, 1932) have found that adults of *C. cilicrura* live for a considerable period and data already given show that in England also the flies may have a long life. The number of days that some captured flies lived during autumn (September-December) is shown below.

♂♂		♀♀	
3 flies lived	10-19 days	...	7 flies lived 10-19 days
4 " "	20-29 " "	...	12 " " 20-29 " "
5 " "	30-39 " "	...	17 " " 30-39 " "
			4 " " 40-49 " "
			2 " " 50-59 " "
			1 fly " 65 " "
			1 " " 79 " "

These periods represent only part of the life of the flies since they were already active when captured and most of the females had been fertilised. Throughout the period (September–December) the flies were kept at temperatures varying from 45° to 63°F. and females laid eggs. Sunshine made them active and unless they were handled with care they escaped from their phials and alighted on the window. Females lived longer than males.

Nematode parasites.

Adults of *C. cilicrura* have been found with nematode parasites which were identified by Dr. T. Goodey as *Heterotylenchus aberrans* Bovien. This species, which is also parasitic on the onion fly, *Hylemyia antiqua* (Mg.), has been described by Bovien (1937). The parasitic worms are remarkable in that parthenogenetic and gamogenetic reproduction alternate in the cycle of generations. Small parthenogenetic females spend their entire lives within the bodies of hosts and give rise to males and females which feed in the reproductive organs and escape by means of the genital aperture. They spend some time as free-living worms, then the fertilised females invade the larvae of the host where, without interfering with its normal development, they continue their development. When mature they give rise to the parthenogenetic generation.

Eggs.

Hawley (1922) found the eggs of *C. cilicrura* on decaying bean pods and haulms, and on rotting cabbage; he also found eggs on newly turned soil and on parched and cracking soil that had been recently watered. Harukawa and others (1930) found eggs in cracks in the soil, especially in newly ploughed fields, and on the food plants, and Rekach (1932) found the eggs in loose damp soil preferably in soil rich in decomposing matter. The writer usually found eggs in the soil at Wye but on one occasion (15th June) found nine eggs on the underside of a healthy cabbage leaf resting on the soil surface.

In captivity, eggs hatched in 2–4 days according to the temperature. Eggs hatched in two days in late spring, summer and early autumn when day temperatures were 60°–70°F.; but in late autumn when night temperatures fell to 40°–45°F. and day temperatures did not exceed 60°F. the incubation period increased to four days. Harukawa and others (1933) found that the incubation period varied from 2 to 17·6 days.

Larvae.

Feeding habits.

Larvae usually feed in the soil but they are sometimes found above ground in the cotyledons and shoots of beans and they have been recorded from the shoots of spinach (Smith, 1933). Captive larvae in glass phials fed on leaf and stem tissue of cabbage and cauliflower and on the cotyledons of peas and beans. The leaf and stem tissue of cabbage and cauliflower was especially satisfactory because the high water content provided the larvae with a moist atmosphere. In order to ensure that the newly hatched larvae quickly became established, the food material was thinly sliced or broken, and consequently it was not discovered whether first-instar larvae were able to feed on unbroken healthy plant tissue. Hawley (1932) thought larvae of *C. cilicrura* showed a preference for decomposing vegetation and Bonde (1939) found them most numerous in disease lesions of potato sets.

Plant tissue given as food to captive larvae decomposed quickly and the larvae fed in the decomposing material, but it is not known whether the decomposition was the result of a natural infection of bacteria or whether it was induced by the

larvae which are known to be vectors of bacterial soft rot (Leach, 1940). Newly hatched larvae seemed incapable of penetrating dry leaf tissue and in captivity they did not penetrate stem tissue except by means of cut surfaces. Broken and decomposing tissue was not essential for third-instar larvae; they tunnelled readily into healthy cotyledons of peas and beans and made holes in the turgid leaves of cabbage.

It appears that the larvae do not depend on living plant material for food because the eggs are commonly laid on bare soil and the incubation period may be completed before germinating seeds or living plants are present. In order to discover how the larvae fed in the absence of living plants, ten eggs were placed (21st August) on the top of each of six beakers (400 ml.) filled with field soil and two beakers filled with straw compost. The eggs hatched (23rd August) and the larvae made their way below the surface but not one of them reached maturity. Again an egg was placed on the top of each of six phials (3 ins. × 1 in.) containing 2 ins. of sifted field soil mixed with approximately $\frac{1}{2}$ sq. in. of shredded cabbage leaf. The eggs hatched (23rd August) and the larvae entered the soil; a small female emerged (23rd September) in one of the phials containing soil and leaf tissue. Shredded cabbage leaf (approximately 6 sq. ins.) was also put in the bottom of a glass jar and covered with a 6-in. layer of sand. Fourteen eggs were placed on the surface (21st September) and hatched in two days (23rd September); after 35 days (28th October) nine small puparia were recovered from the sand and the first adult (♀) emerged on 9th February.

These experiments demonstrated that the larvae were able to complete their development on decaying vegetable matter in the soil and suggested that in the field they were primarily scavengers. It had been previously observed (Miles, 1948) that larvae fed on the buried residues of a crop of cabbage seedlings. Harukawa and Kumashiro (1930) also found that larvae of *C. cilicrura* were able to complete their development without living plant material. In their experiment larvae reached maturity in soil containing cotton-seed meal, an organic fertiliser.

Activity in the soil.—The larvae showed a marked tendency to wander. Newly hatched larvae on suitable food wandered for some hours before settling down to feed, and similar larvae on the top of a 6-in. layer of sand found their way to leaf tissue buried beneath.

Exhaustion of the food supply induced great activity in third-instar larvae. Larvae in a jar of sand with an inadequate supply of food were so active that the sand in contact with the sides of the jar became closely covered with a network of larval tunnels and the larvae were frequently seen making their way through the sand.

Larvae in all stages moved away quickly from light thus ensuring that they sought their food in the soil instead of wandering on the surface. Larvae kept in phials without soil fed within the tissue or under it.

Duration of stages.—Larvae of *C. cilicrura* have three instars, the duration of which depends on temperature and availability of food. Observations on the development of larvae from three batches of eggs gave the following data :—

Egg batches	Duration in days			
	1st stage	2nd stage	3rd stage	Total
No. 1	3	3	6-8	12-14 days
No. 2	3	3	5-7	11-13 days
No. 3	3	3	6-10	12-16 days

These larvae hatched on 22nd and 23rd May and fed until 3rd to 7th June; consequently they developed under similar temperature conditions (the mean

maximum temperature for the period was 61.6°F. and the mean minimum temperature 43.4°F.). Earlier (10th to 20th May), when the mean maximum temperature was 68.2°F. and the mean minimum temperature 49.9°F. larvae completed their development in eight to ten days. Larvae kept at laboratory temperatures during October and November required 15 to 24 days to complete their feeding period.

Puparia.

The pupal stage is passed in the soil. Larvae reared in captivity without soil started to wander when they had finished feeding and when offered soil or sand they burrowed into it immediately.

No measurement of the depth at which pupation normally takes place has been made, but in fields infested with *C. cilicrura* puparia have been found in the top three inches of soil. When captive larvae were fed on vegetable matter buried six inches below the surface puparia were subsequently found one to two inches below the surface.

The period between the mature larva entering the soil and the emergence of the imago has been used to indicate the length of the pupal period; for 18 larvae that entered the soil 17th May to 7th June it was as follows:—

No. of larvae	Duration of pupal stage
1	16 days
1	17 "
4	18 "
2	19 "
6	20 "
4	21 "

Larvae that entered the soil in October and November and were kept at room temperatures (43–64°F.) had pupal periods ranging from five weeks to over five months. This suggested that larvae reaching maturity late in the season tend to have a pupal diapause. When kept in an insectary, larvae that reached maturity in the late autumn hibernated as puparia and emerged in early spring. Occasionally flies emerged during the winter months. In 1949 a male emerged on 19th January, after a pupal stage of 14 weeks, and another on 10th February after 17 weeks in the soil. Similar variability in the duration of the pupal period has been observed by Harukawa and others (1930, 1933), Rekach (1932), and Reid (1940).

Discussion.

The study of the habits of adults and larvae of *C. cilicrura* suggests that the larvae are primarily scavengers feeding on organic matter in the soil, and that attacks on crops may be associated with a lack of natural food. The incidental nature of attack seems to account for the wide range of recorded host plants. The larvae are well known in parts of Europe, Asia, North Africa, and South America as parasites or predators in the egg-capsules of locusts and grasshoppers and it seems probable that this carnivorous habit is also incidental and associated with a lack of other food. Eberhardt (1930), who recorded 8 to 60 larvae of *C. cilicrura* in the egg-capsules of migratory locusts in Daghestan, noted that the flies swarmed on the soil in warm sunny weather when digging for locust egg-capsules was in progress.

Fuller knowledge of the habits of the adults and larvae should lead to a better understanding of the problem of control. The date of preparation of the seed beds seems important since in England peas, usually sown in March, are not often attacked, while the seed beds of runner and dwarf beans, usually prepared about mid-April, are often heavily infested.

The relationship between the use of organic fertilisers and the incidence of attack is not yet clearly understood. Investigators (Rekach, 1932; Harukawa & others, 1930; Reid, 1940) have found that the application of certain organic fertilisers such as cotton-seed meal and fish meal, increases the attraction of the soil for the flies. As they also provide alternative food for the larvae (Harukawa & others, 1930; Reid, 1940) the use of these substances might be expected to mitigate attack on the seedlings. The habits of the flies show that the design and conduct of experiments involving the use of manures need careful planning, for the day-to-day concentration of flies on experimental plots is affected by weather conditions and by cultural operations in progress in surrounding areas.

The habits of the flies show also the importance of timing control measures if they are to serve their intended purpose. Repellents against egg-laying adults should be applied simultaneously with the operations that disturb the soil otherwise they are not likely to be effective. Egg-killing substances should be applied within two days of disturbing the soil in view of the fact that the eggs have a short incubation period. Substances intended to destroy the larvae should also be applied within two days of disturbing the soil otherwise the larvae may have penetrated beyond the range of the toxic dressing in search of food; their toxicity should be sufficiently lasting to affect older larvae that wander towards the surface as the supply of buried food becomes exhausted.

II. THE BIOLOGY AND ECONOMIC STATUS OF *Pegohylemyia fugax* (Mg.).

Larvae of *Pegohylemyia fugax* (Mg.) have been taken on a wide range of crops, sometimes in considerable numbers, in the course of investigations on the bean seed fly. Since pathological conditions were present in crops from which *P. fugax* was collected and there was little information regarding the species, it seemed advisable to collect data on its biology and economic status.

Occurrence on cultivated Plants.

P. fugax has been recorded on a number of cultivated plants. Professor Herbert W. Miles (1926) bred it from heads of cauliflowers in Lincolnshire, and Dr. K. M. Smith (1927) appears to have found it while he was working on *Chortophila brassicae* (Behé.) because the unidentified dipterous larva with one bifid tubercle shown in fig. 7 of his paper is undoubtedly *P. fugax*, and not *C. pilipyga* (Villen.) as he thought. More recently, it was recorded (Min. Agric. Monthly Summaries) that specimens were bred from cauliflower heads (September, 1945), and from brussels sprouts (December, 1948). Mr. J. F. Perkins, of the British Museum (Natural History), states (in correspondence) that he has bred the species from oat seedlings.

P. fugax is widely distributed in Europe. In Holland, van Poeteren (1925) recorded that carnations were mined by the larvae and again (1931) that the species occurred on beet. Bruneteau (1930) found it associated with mines in the leaves of carnations in France. Lundblad (1933) recorded it as occurring locally in Sweden in small numbers on brassicas, whilst in North America, Frost (1924) associated this species with leaf-mines in beet, spinach and several weeds.

During the period 1945-1948, the writer took larvae of *P. fugax* from summer cabbages, sweets, cabbage seedlings, spring cabbages, turnips, lettuce, cauliflowers and brussels sprouts.

Adult.*

The flies are greenish-grey and about 5 mm. long. The males and females differ in appearance as in the case of other species of the group *Chortophila*. Males are

* Representative adults were identified as *P. fugax* by Mr. J. E. Collin.

dark greenish-grey with a broad dark median stripe and somewhat indistinct lateral stripes on the thorax and a dark median stripe on the abdomen. The females are light greenish-grey with the dark stripes narrow and indistinct. The legs are dark in both sexes. The wings are clear grey with brownish-grey nervures. The costa which extends to the medius is fringed with short spines and has a longer spine at the junction of the sub-costa. The medius is nearly straight and the first anal vein extends to the margin of the wing.

The head is dark greenish-grey in the males and lighter in the females. The eyes are without microscopic hairs. In the males, the eyes are separated by a narrow stripe, but in the females there is a light grey frontal stripe about one-third of the width of the head and an orange yellow area extending from the lunule to the depression surrounding the ocellar triangle. The arista is sparsely pubescent, the proboscis slender, and palpi dark.

The thorax is about the same width as the head and is rather convex dorsally. Important features of the chaetotaxy are found on the thorax and legs. The pre-alar (first supra-alar) is about one-third as long and thick as the second supra-alar. Males and females differ in the character of the lower hind sterno-pleural bristle; in the males it is almost as long and stout as the upper hind sterno-pleural bristle, but in females it is only about one-quarter of this size. The tibia of the mesothoracic leg in both sexes has one strong antero-dorsal bristle, two postero-dorsal bristles, and no antero-ventral bristles; the first tarsal segment has no long setae on the outside and the second tarsal segment is not swollen on the inner surface. In both sexes, the femur of the metathoracic leg is without strikingly long ventral bristles. The metathoracic tibia has a series of 5-6 (in females) or 6-7 (in males) antero-dorsal bristles uneven in length and irregularly spaced; 2-3 (in females) or 3-4 (in males) antero-ventral bristles somewhat strong but slightly irregular in length and mainly in the apical half of the segment, and in the males there is a series of 6-7 rather irregular postero-ventral bristles in the basal half of the segment.

The abdomen presents no distinctive taxonomic characters. The dark median stripe is distinct in males and indistinct in females. The hypopygium and ovipositor are inconspicuous. The glistening greenish-grey colour is distinct and characteristic of the species.

Habits.—Flies were captured at Fladbury, Worcs., and Wye, Kent, on cabbages, cauliflowers, brussels sprouts, and lettuces, and they were also found on small heaps of weeds and rakings in the last-named locality. They were active in sunshine but appeared to shelter in vegetation when the weather was dull, occurring in greatest numbers in May and in mild sunny weather in September and October. In captivity they fed readily on dilute sugar solution and lived up to 25 days.

Egg-laying.—No data are available concerning the egg-laying capacity of *P. fugax*, as bred flies were not fertilised in captivity and no eggs were produced. A captured fly laid 11 eggs on the third day after capture, 9 eggs on the sixth day and 37 eggs on the eighth day, making a total of 57 eggs; another laid 16 eggs on the second day and 28 eggs on the ninth day, making a total of 44 eggs.

The eggs have been found on cabbages, cauliflowers, brussels sprouts and lettuces in May, September and October. They occurred singly and in small batches of 2-6 on the backs of leaves, in leaf axils and on dead leaves at the base of the plants. They were also found in large batches of 40-50 in cracked and broken stems, on tissue affected by soft rot, in wounds caused by cabbage stem weevil, *Ceuthorrhynchus quadridens* (Panz.), and on the cut stems of cabbages and cauliflowers after harvesting.

The incubation period at room temperatures in the laboratory in September and October was 2-4 days.

Larva.

Larvae have been found among the leaves and within stems of cabbages, in the "curd" of cauliflowers, in the crowns of turnips and swedes, and in lettuces. They have been reared in the laboratory on decomposing leaf and stem tissue of cabbage and cauliflower, the feeding period in September and October being 10 to 16 days.

Fully grown larvae are 8–9 mm. long, rather slender and translucent white. The body tapers towards the vestigial head and is truncated posteriorly; the flat area surrounding the posterior spiracles has a conspicuous corona of large conical tubercles with a median bifid tubercle on the ventral edge (figs. 1 and 2). The larval skin is closely covered with minute transparent spicules that give it a woolly appearance when viewed under the microscope. The texture of the skin, the conspicuous corona of large conical tubercles, and the median posterior bifid tubercle make larvae of *P. fugax* readily recognisable.

Parasites.—In the autumn of 1947, hymenopterous parasites were bred from larvae of *P. fugax*; these were identified by Mr. G. J. Kerrich, Commonwealth Institute of Entomology, as *Pseudeucoila* sp. near *cubitalis* (Hartig).

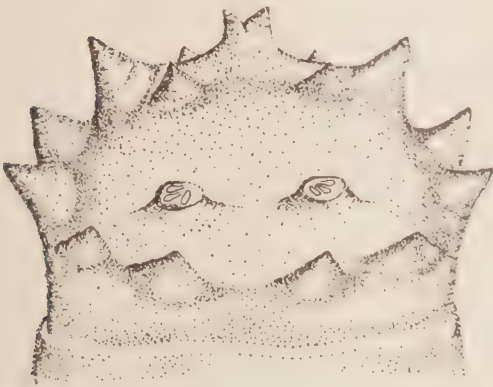


Fig. 1.—Caudal segment of larva of *P. fugax*—Dorsal view ($\times 40$).

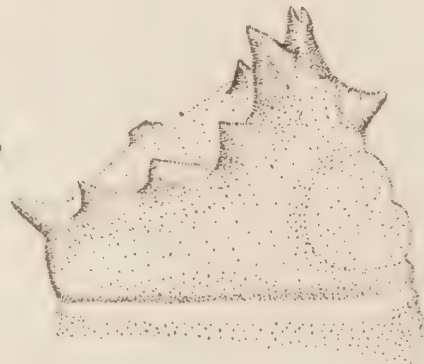


Fig. 2.—Caudal segment of larva of *P. fugax*—In profile ($\times 34$).

Puparium.

Larvae in captivity tunnelled into soil to pupate. Wild puparia have been found in the soil at the roots of cauliflowers, swedes and turnips in company with those of cabbage root fly (*C. brassicae*). Apparently some larvae of *P. fugax* pupate amongst the leaves in which they have been feeding because numbers of puparia were found in brussels sprouts in December 1948.

The puparia are 5 mm. long, dull dark brown with the intersegmental lines sharply defined and the larval corona clearly visible as a circlet of stout projections. The hardening and shrinking of the larval integument with its spicules give the puparia a rough surface to which particles of soil adhere. The dark colour and the adherence of soil particles make it difficult to find them in the soil.

The duration of the pupal period varied with the temperature and the time of the year. Captive larvae entered the soil on 24th May and emerged on the 14th and 15th June after a pupal period of 21 or 22 days. Larvae that entered the soil during

the last week of October began to emerge on 27th November and continued until mid-February. The winter is passed in the pupal stage under natural conditions.

Adults have been taken at various times from May to late October and larvae have been found from mid-May to mid-December. As the cycle from egg to adult may be completed in 4-5 weeks, it is probable that there are at least four generations a year.

Economic Status.

Observations on the occurrence and feeding habits of *P. fugax* have continued over three seasons. The species was first found on cabbage at Long Ashton, Somerset, in August and September 1945. Larvae were found in October 1945 in the crowns of swedes at Chippenham, Wilts., in association with larvae of cabbage root fly and among cabbage seedlings at Fladbury, Wores., in association with larvae of the bean seed fly (*C. cilicrura*). Adults, eggs and larvae were present in large numbers in May 1946 on spring cabbages at Fladbury, Wores. At Wye, Kent, they were found in the crowns of turnips and swedes in company with larvae of cabbage root fly in September and October 1947; they were also found among lettuce. They were numerous in the hearts of brussels sprouts in November and December. Larvae and pupae were collected from brussels sprouts and cauliflowers in November and December 1948. Pathological conditions were always present in plants infested with larvae of *P. fugax*.

Larvae of *P. fugax* were always found in an environment which included one or more of the following primary parasites: cabbage root fly larvae, mealy cabbage aphid, *Brevicoryne brassicae* (L.), bacteria (associated with soft rots in crucifers), botrytis and other fungi (associated with disease in lettuce and crucifers). Predatory Anthomyiids (larvae of *Phaonia*, etc.), mycophagous larvae of fungus gnats (*Sciara* spp.) and saprophytic eelworms (*Rhabditis* spp., etc.) were also present.

Attempts to rear larvae to maturity in the autumn of 1945 were unsuccessful. In May 1946 and again in the autumn of 1948, eggs and immature larvae were successfully reared to maturity on leaf and stem tissue of cabbage infected with soft rot. The larvae seemed unable to feed on healthy tissue but survived in isolation on rotting tissue. They were not generally cannibalistic and several could be fed together in small phials. It was concluded from experimental rearing that the larvae were saprophytic, and that pathological conditions in plants infested with *P. fugax* were produced by other organisms.

Analysis of the circumstances in which larvae of *P. fugax* have been recorded confirmed the view that they were saprophytic. Their presence was usually associated with phytophagous species. Bruneteau (1930) recorded it on carnations with *Hylemyia*, *H. cardui* (Mg.) and *H. brunescens* (Zett.), which tunnel in the leaves and shoots of carnations and whose attack would create conditions suitable for the feeding of saprophytic species. Similarly it has been recorded from North America (Frost, 1924) and Holland (van Poeteren, 1931) in the leaves of beet and spinach in association with *Pegomyia betae* (Curt.). Perkins (in correspondence) found it in decomposing oat seedlings which had been attacked by frit fly, and its presence in the heads of cauliflowers (Min. Agric., 1945) was associated with the occurrence of *C. brassicae*.

The writer has always found *P. fugax* in association with parasitic organisms. In the crowns of swedes and turnips, and in stems of swedes, cabbages and cauliflowers it has been associated with larvae of cabbage root fly and cabbage stem weevil. It occurred in the stems of cabbage seedlings also attacked by *C. cilicrura*. Spring cabbages affected by bacterial soft rot harboured large numbers of the species. Lettuce attacked by *P. fugax* showed extensive decomposition as a result of *Botrytis*,

and its occurrence in brussels sprouts was simultaneous with the occurrence of larvae of cabbage root fly and fungi, or followed injury by mealy cabbage aphids.

Summary.

Observations have been made on the life-history and habits of *C. cilicrura*. The flies came to moist, freshly turned soil at all times from April to October, particularly in warm bright weather, but the number of flies seen on observation plots was affected by the area of damp and disturbed soil nearby. Both males and females were attracted and some females laid eggs within a few minutes of their capture. When isolated in phials and fed on dilute sugar solution, captured flies lived up to 79 days. Captured females spent varying periods up to 44 days before laying eggs. The eggs were usually laid in batches of less than ten at irregular intervals over periods extending up to 28 days, and 53 eggs per fly was the highest number recorded. Parasitism by the nematode, *Heterotylenchus aberrans* Boven, occurred.

Eggs were found in soil crevices and on vegetation in contact with soil. The observed incubation period was 2-4 days according to the temperature.

The duration of the larval instars was affected by temperature: in captivity, the larval feeding period was 8-16 days in May-June and 15-24 days in October-November. The pupal stage lasted from 16-21 days in May-June and from five weeks to five months in autumn and winter. A few flies emerged during January and February in the insectary.

In captivity first-instar larvae found food buried at a depth of 6 ins. and they were able to reach maturity on fragments of vegetation buried in sand. It is suggested that they are primarily scavengers on organic matter in the soil.

Larvae of *P. fugax* have been found in the stems and crowns of turnips and swedes, and in cabbages, cauliflowers, lettuces and brussels sprouts. A study of their habitat shows that they occur in decomposing vegetation.

Eggs have been found about various brassicas. Their incubation period was 2-4 days. Larvae were reared in decomposing vegetation. Pupation takes place in the soil and in the feeding sites. Adults have been taken from May to October. The life-cycle from egg to adult occupied 4-5 weeks in spring and the winter was passed in the pupal stage.

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References.

- BONDE, R. (1939). The role of insects in the dissemination of potato blackleg and seed-piece decay.—*J. agric. Res.*, **59**, pp. 889-917.
- BOVIEN, P. (1937). Some types of association between nematodes and insects.—*Vidensk. Medd. dansk naturh. Foren.*, **101**, pp. 1-114.
- BRUNETEAU, J. (1930). La mouche de l'oeillet, *Hylemyia brunnescens*, Zetterstedt.—*Rev. Zool. agric. appl.*, **29**, pp. 37-46.
- *LEBERHARDT, G. (1930). *Chortophila cilicrura* Rond., a new parasite of the migratory locust in Dagestan. [*In Russian.*]—*Plant Prot.*, **6**, pp. 813-814. (R.A.E., (A) **19**, p. 50.)
- FROST, S. W. (1924). A study of the leaf-mining Diptera of North America.—*Mem. Cornell agric. Exp. Sta.*, no. 78, 228 pp.

- *HARUKAWA, C. & KUMASHIRO, S. (1930). Studies on the Seed-corn Maggot, *Hylemyia cilicrura* Rondani, in Japan. I.—Ber. Ohara Inst. landw. Forsch., **4**, pp. 371–382. (R.A.E., (A) **18**, p. 675.)
- *HARUKAWA, C., TAKATO, R. & KUMASHIRO, S. (1933). Studies on the Seed-corn Maggot, II.—*Ibid.*, **5**, pp. 457–478. (R.A.E., (A) **21**, p. 402.)
- HAWLEY, I. M. (1922). Insects and other animal pests injurious to field beans in New York.—Mem. Cornell agric. Exp. Sta., no. **55**, pp. 943–1037.
- LEACH, J. G. (1940). Insect transmission of plant diseases.—615 pp. New York, McGraw Hill.
- LUNDBLAD, O. (1933). Källflugorna. Om några i de odlade kålväxternas rot- och stamdelar levande flugarter, särskilt med hänsyn till större kålflugen (*Hylemyia floralis* Fall.).—Medd. St. Växtskyddsanst., no. **3**, 103 pp.
- MILES, H. W. (1926). Agricultural entomology of Holland Division of Lincs.—Trans. Lincs. Nat. Un., 1926, pp. 129–148.
- MILES, M. (1948). Field observations on the bean seed fly (seed corn maggot), *Chortophila cilicrura*, Rond., and *C. trichodactyla*, Rond.—Bull. ent. Res., **38**, pp. 559–574.
- *VAN POETEREN, N. (1925). Verslag over de Werkzaamheden van den Plantenziektenkundigen Dienst in het Jaar 1924.—Verslag. & Meded. Plantenziektenk. Dienst, no. **41**, 62 pp. (R.A.E., (A) **13**, p. 362.)
- *VAN POETEREN, N. (1931). Verslag over de Werkzaamheden van den Plantenziektenkundigen Dienst in het Jaar 1930.—*Ibid.*, no. **64**, 189 pp. (R.A.E., (A) **19**, p. 739.)
- REID, jr., W. J. (1936). Relation of fertilizers to Seed Corn Maggot injury to spinach seedlings.—J. econ. Ent., **29**, pp. 973–980.
- REID, jr., W. J. (1940). Biology of the Seed-corn Maggot in the coastal plain of the South Atlantic States.—Tech. Bull. U.S. Dep. Agric., no. **723**, 43 pp.
- *REKACH, V. N. (1932). Studies on the biology and control of the Corn-seed Maggot (*Chortophila cilicrura*, Rond.). [In Russian.]—Trud. Zakavk. nauchno-issled. khopk. Inst., no. **16**, 26 pp. (R.A.E., (A) **20**, p. 349.)
- SMITH, C. E. (1933). An unreported habit of the Seed Corn Maggot, *Hylemyia cilicrura*, Rond.—J. econ. Ent., **26**, pp. 910–911.
- SMITH, K. M. (1927). A study of *Hylemyia* (*Chortophila*) *brassicae* Bouché, the Cabbage Root Fly and its parasites. . . .—Ann. appl. Biol., **14**, pp. 312–330.

THE COTTON JASSID, *EMPOASCA LYBICA* (DE BERG.) DURING THE DEAD SEASON⁷ IN THE GEZIRA, ANGLO-EGYPTIAN SUDAN.

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It is the general agricultural practice in the Gezira that, after the picking season, the cotton sticks are pulled up (about the middle of May), collected into heaps and burnt. Motoring through the Gezira after the cotton is pulled out, one cannot help noticing the absence of vegetation.

It was of great economic importance to ascertain where the Jassids live after the cotton has been removed as it would save a great deal of expense if the insect could be exterminated during the dead season.

At first it was thought that the Jassids may aestivate remaining dormant in the soil until the cotton is sown in August, when they migrate to it. The fact that all stages (eggs, nymphs and adults) could be found on different plants in large numbers during the dead season excluded this possibility. In addition, the integument of the Jassid is so thin that it does not stand desiccation even for a short time.

During a survey undertaken in the Northern Gezira at fortnightly intervals during the dead season (May and June), Jassids were found breeding, or living as adults, on certain vegetables and weeds in the gardens and along the river bank. No Jassids could be found elsewhere as tenancies were devoid of vegetation and the canal banks did not support the kinds of weed favoured by them.

The following host plants were found to support all stages of cotton Jassids during the dead season: *Solanum dubium* Fresn., *Hibiscus esculentus* L., *Abutilon glaucum* Webb, *Solanum melongena* L., *Psidium guajava* L., *Rhynchosia memnonia* DC., *Ricinus communis* L., *Helianthus annuus* L., *Hibiscus sabdariffa* L., *Cucurbita pepo* L., *Vicia faba* L., *Lawsonia alba* Lam., and *Ziziphus spina-christi* Lam.; whilst the following supported adults only: *Dolichos lablab* L., *Medicago sativa* L., *Vigna unguiculata* (L.) Walp., *Lycopersicum esculentum* Mill., *Ipomoea cordofana* Choisy, *Gisekia pharnacioides* L., *Corchorus olitorius* L., *Acacia arabica* Willd., *Canna indica* L., *Euphorbia* sp., *Citrus medica* var., *Azadirachta indica* Ajuss., *Pithecolobium dulce*, *Berith*, and *Balanites aegyptiaca* Del.

The Egyptian bean (*Vicia faba*) is occasionally grown during the winter in native gardens and this plant invariably maintains a large population of *Empoasca lybica*. All stages of this Jassid occur at times on castor oil (*Ricinus communis*) but the season of abundance appears to coincide with that on cotton. Small numbers bred on guava trees as soon as new growth developed at the beginning of the rains. *Acacia arabica* also supports a small number of adults but no nymphs could be found.

Host Selection.

A number of vegetables and weeds at Soriba house garden (Central Gezira) were examined on 14th July 1947 to determine whether any marked preference for certain hosts were exhibited, with the following results expressed as nymphs per 100 leaves, *Solanum melongena* 540, *S. dubium* 497, *Hibiscus esculentus* 413, *Abutilon glaucum* 190, *Corchorus olitorius* 0.

Movement of Jassids to Cotton.

A sticky screen with four surfaces each measuring 1 metre square was erected at the beginning of August on four poles 16 ft. high to catch migrating insects. No Jassids were caught during the rains but, of those trapped in the middle of November, 17 were caught on the south side, four on the north, seven on the east, and eight on the west side.

The above figures indicate that the initial movement to cotton is very small, and that the build-up of numbers takes place within the cotton crop.

The numbers of Jassid adults and nymphs from 200 plant holes from cotton "numbers" (*cf.* Cowland & Edwards, 1949, p. 91) adjacent to gardens in the Northern Gezira for comparison with those from numbers further away are given in Table I.

TABLE I.

Date	No. of Nymphs		No. of Adults		Total	
	A	B	A	B	A	B
September						
11	74	7	6	6	80	13
11	139	75	61	19	200	94
13	193	56	61	12	254	68
14	148	52	11	5	195	57
14	457	151	50	27	507	178
15	988	444	56	48	1,044	492
16	307	12	42	19	349	31
17	712	157	186	33	898	190
18	367	71	52	30	419	101
18	1,012	357	81	61	1,093	418
20	203	145	31	23	234	168
22	652	312	76	52	728	364
22	643	107	134	54	777	161

A, nearest cotton to house gardens as a source of infestation.

B, furthest cotton from house gardens as a source of infestation.

It will be seen that larger numbers occurred nearest the house gardens, the average being :—

	Nymphs	Adults
Nearest the house gardens	456	66
Farthest from house gardens	150	30

Table II shows the difference between the first four angias* nearest and the last four angias farthest from some of these house gardens.

*A tenancy (hosha) is 10 acres and is divided into 14 or 16 sections (angias) for watering purposes.

TABLE II.

Date	No. of Nymphs		No. of Adults		Total	
	A	B	A	B	A	B
September						
11	74	0	5	0	79	0
13	220	132	10	7	230	139
14	172	5	10	1	182	6
14	199	90	24	13	223	103
17	362	262	76	66	438	328
24	360	78	64	7	424	85
24	599	315	30	18	629	333
24	498	346	39	16	537	362
24	589	316	30	18	619	334
27	683	293	65	23	748	316

A, nearest cotton to house gardens as a source of infestation.

B, furthest cotton from house gardens as a source of infestation.

The first four angias had an average of 367 nymphs and 35 adults and the last four 184 nymphs and 17 adults showing that the angias nearest the gardens had more Jassids than those further away.

Spraying the Gardens during the Dead Season.

From the foregoing observations, it was clear that the house gardens harbour Jassids during the dead season but that when the cotton germinates, they migrate to it and cause reinfestation.

An attempt was made to control the insect during the dead season in the Northern Gezira and all gardens to the west of the main canal in Laota, Kab El Gidad and Meilig Blocks as far south as Badr canal, were sprayed with 0.1 per cent. DDT emulsion on 15th to 17th of June 1947 leaving the gardens on the east side of the main canal unsprayed for control.

Although no Jassids were found immediately after spraying they reappeared on the vegetables and weeds a week or so after spraying.

Five cotton "numbers" on both the eastern and western sides of the main canal, all sown on the 20th August, were examined on 9th November 1947. The average number of nymphs per 100 leaves during the peak of average infestation was found to be 1,312 nymphs on the eastern side of the main canal and 938 on the western side.

The treated side had therefore 28.5 per cent. nymphs less than the untreated one.

As the foregoing result was not satisfactory, another attempt was made in the dead season of 1948. The above-mentioned gardens were sprayed three times, at weekly intervals, with 0.5 per cent. DDT emulsion. The sunt trees (*Acacia arabica*), which were not sprayed in the 1947 season, were also treated as they had been found to support the adult cotton Jassid.

No Jassids appeared immediately after spraying but within a few days the sprayed plants were found to contain adults which seemed to have migrated from the tops of *Acacia* trees which had not been reached by the spray.

About the middle of November 1948, six " numbers " of cotton were examined on each side and 275 nymphs per 100 leaves were found on the eastern side and 228 on the western.

This reduction of about 17 per cent. in the number of Jassids in the western side, due to spraying, was not appreciable.

Without complete or almost complete extermination of Jassids during the dead season, on the small amount of vegetation that is present, spraying will still be necessary on the succeeding cotton crop since the rate of Jassid increase is very great under post-rain conditions. The poor results obtained from spraying the gardens during the dead season may be due to the difficulty of spraying tall acacia trees satisfactorily and of covering both sides of the low growing weeds under which Jassids may be breeding. (The chemicals used for indicating the presence of DDT on the leaves did not arrive in time for trial in case the spray may have been at fault.)

Summary.

Regular surveys were carried out from May to July to find out where Jassids live during the cotton close season.

A list of host plants on which Jassids can breed, or feed only as adults, is given; they exhibit preference for certain plants.

The movement of Jassids to cotton was recorded. The initial infestation of cotton is very small but is rapidly built up within the cotton crop. Gardens are shown to be the chief sources of infestation and cotton fields nearest them show larger initial numbers.

Attempts to exterminate Jassids in these gardens by spraying all plants with 0.1 per cent. or 0.5 per cent. DDT emulsion during the summer dead season were not successful. It is thought that reinfestation came from adults which had been present on the tops of *Acacia arabica* trees and had not been reached by the spray.

Acknowledgements.

The authors wish to express their thanks to the Manager and Inspectors of the Sudan Plantations Syndicate for their co-operation.

Reference.

- COWLAND, J. W. & EDWARDS, C. J. (1949). Control of *Empoasca lybica*, de Berg., on Cotton in the Anglo-Egyptian Sudan.—Bull. ent. Res., **40**, pp. 83-96, 1 pl., 3 figs.
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THE EFFECT OF RAINFALL ON THE COTTON JASSID, *EMPOASCA LYBICA* (DE BERG.) IN THE GEZIRA, ANGLO-EGYPTIAN SUDAN.

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The Gezira Irrigation Scheme draws its water from the Sennar Dam on the Blue Nile. It lies in a triangle of which the apex is Khartoum and the sides consist of the converging White and Blue Nile rivers. The Scheme stretches for about 100 miles along the West bank of the Blue Nile from Masid in the North (Lat. $15^{\circ}16'N$.—Long. $32^{\circ}57'E$.) to Hag Abdalla in the South (Lat. $13^{\circ}58'N$.—Long. $33^{\circ}35'E$.).

The land is divided up into Blocks (each from 12,000–35,000 feddans*), a quarter of each block being under cotton annually. A block is administered by an Inspector-in-charge assisted by one or more Inspectors living in houses situated within the area. Each house has a garden of 5–10 feddans in which vegetables, fruits, flowers and large numbers of shade trees are grown. Except near the river, these gardens have practically the only trees in the area and during May and June they form islands of green vegetation in a large dry area.

The crops grown in the Scheme are mainly cotton and dura (millet), the latter being sown during July and early August. The land to be planted with cotton is watered early in August and sown between the 10th and 31st of the same month.

There are two varieties of cotton grown in the Gezira :—

(1) Sakellaridis, a high-grade staple cotton usually sown in the Northern part of the Gezira.

(2) X1730, a selected variety, yielding a medium-grade cotton, usually sown in the Southern Gezira as it is resistant to leaf curl disease which is prevalent there.

The cotton is planted and established during the rainy season which lasts from 15th July to 15th October.

The main pest in the Gezira is the cotton Jassid, *Empoasca lybica* (de Berg.) which sucks the sap from the leaves causing them to turn a reddish colour and curl up. Shedding of leaves may take place and the crop is reduced considerably.

The Gezira Scheme can be divided into three main areas in respect of the incidence of cotton Jassids :—

The Northern area of normal abundance, from Masid to Abu Usher, (Lat. $14^{\circ}55'N$.—Long. $33^{\circ}11'E$.), in which the insect is always numerous during the cotton season and usually causes heavy damage.

A Central area of occasional abundance from Abu Usher to Kilo 77 (Lat. $14^{\circ}20'N$.—Long. $33^{\circ}25'E$.) where Jassids are always present but only occasionally reach pest proportions.

A Southern area of possible abundance from Kilo 77 to Hag Abdalla where the pest is present in small numbers only and rarely causes damage.

Five cotton " numbers " (90 feddans each) (see Cowland & Edwards, 1949, p. 91) were examined in each area about the 11th November, 1947, to confirm that the population of Jassids in these areas differs. The average number of nymphs per 100 leaves (five leaves were taken from each plant, one from the top, another from the bottom and three alternating ones from the middle) was found to be 1,125 nymphs in Area I, 650 in II and 139 in III.

*A feddan is 1.038 acres.

This difference in the number of Jassids in the three areas might be due to the factors discussed below.

Nutritional Factors.

Water content.

The average water content of cotton leaves collected, as indicated above, about the 15th November 1947 at 11 a.m. from the three areas was 74.5 per cent. in Area I, 75.9 per cent. in Area II and 75.6 per cent. in Area III showing that there was no appreciable difference in the three areas.

Nitrogen content.

Data in the files of the Plant Physiologist of the Research Farm, concerning the nitrogen analysis of the leaves of Sakel cotton grown in the North of the Gezira and of X1730 in the South, showed that the latter has a higher nitrogen content (about 1 per cent. more). A high nitrogen content is known to increase the output of egg laying in insects. Consequently, if this factor was operating, more Jassids would be expected in the South than in the North.

Carbohydrates.

Owing to lack of equipment it has not been possible to secure any data on the carbohydrate content of the leaves.

Mechanical Factors.

Sections of the leaves from Northern and Southern Gezira cotton did not exhibit any apparent difference in the thickness of the epidermis.

The Variety of Cotton.

The average number of nymphs counted per 100 leaves of the Sakel and X1730 grown side by side is shown in Table I.

TABLE I.

Locality	Date of counting	No. of Nymphs on Sakel	No. of Nymphs on X1730
Research Farm	5.xi.1948	344	324
Mesellemiya Group	10.xi.1948	777	773

The difference in the population of Jassids could not therefore be due to the variety of cotton.

Biotic Factors.

Vegetational succession.

The fortnightly survey of the Northern Gezira in 1947 and 1948 showed that the Jassids live mainly, during the dead season (from April to June) on some trees, vegetables and weeds mainly in the gardens of the Inspectors and on the river bank. When the rainy season starts, weeds, especially gebbein (*Solanum dubium* Fresn.) and hambuk (*Abutilon glaucum* Webb) which are favourite hosts before the cotton is sown, appear everywhere, especially on the fallow land. If the rains are poor the germination of the seed of these weeds is adversely affected but, on the other hand,

heavy rainfall produces an abundance of them. In 1947, there were poor rains in the Northern Gezira and in consequence, they were scarce on the fallow land, but in spite of this the number of Jassids on the cotton was very high. Owing to heavy rain in the Southern Gezira, where the Jassid infestation is slight, these weeds appeared in great quantities.

Predators.

The larvae and adults of *Coccinella rufescens* (Muls.), *Cydonia vicina* (Muls.) and *Exochomus nigromaculatus* (Goeze) were found to seek and devour the nymphs.

The larvae of *Chrysopa vulgaris* Schneider and also some spiders that have not yet been identified were also found to feed on the nymphs.

No outstanding difference in the numbers of these predators could be detected in the three areas mentioned above.

Effect of Rain on Jassids.

Meteorological data for the Northern and Southern part of the Gezira show that the difference in temperature and humidity is not very great, but that there is a striking difference in the amount of rainfall.

If the peak incidence of adult Jassids (Cowland, 1947) caught by making 96 double sweeps with a net on cotton in Turabi (Northern area) and the Gezira Research Farm (Middle area) is plotted against the total rainfall, a slight correlation appears which becomes more significant when the rain in July and August only is taken. If, however, only showers of over 10 mm. in July and August are considered the correlation is very striking. The outstanding constant feature in figs. 1 and 2 is the decrease in the number of Jassids in the year of heavy rainfall (shower of over 10 mm.) in July and August and *vice versa*.

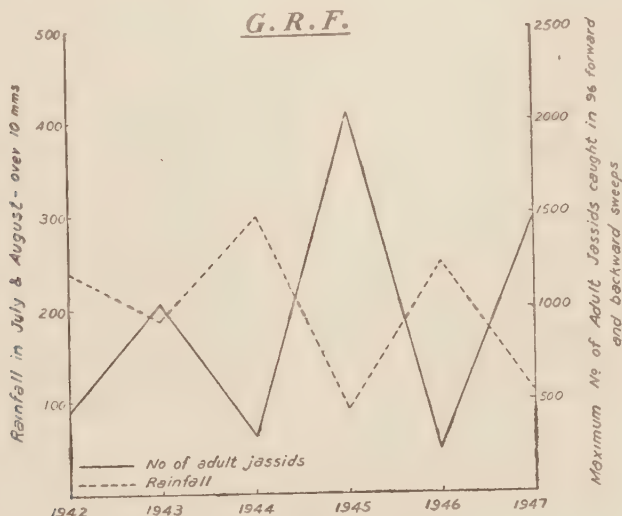


Fig. 1.

About half an acre of cotton in Haram Sereiha (North area) was heavily sprayed twice with water on 28.ix.1947. The average number of nymphs per 100 leaves before and after spraying was 266 and 228 respectively giving a reduction of only 14.2 per cent. which was not appreciable.

If the under surface of the leaves of weeds and cotton are examined during the rainy season, particles of mud can be seen to be adhering to them. It seems that the effect of rain is to splash mud on to the under surface of the leaves. It was, therefore, decided to spray about half a feddan of cotton in the Gezira Research Farm with water to which fine dust was added (2 kgs. per 4 gallons of water on 15.xi.1947) to ascertain whether these particles of mud have any effect on Jassids. The number of nymphs per 100 leaves before and after spraying was 303 and 43 respectively—a reduction of 85.8 per cent. After a second application of spray the number was further decreased to 18. A total reduction of 94 per cent.

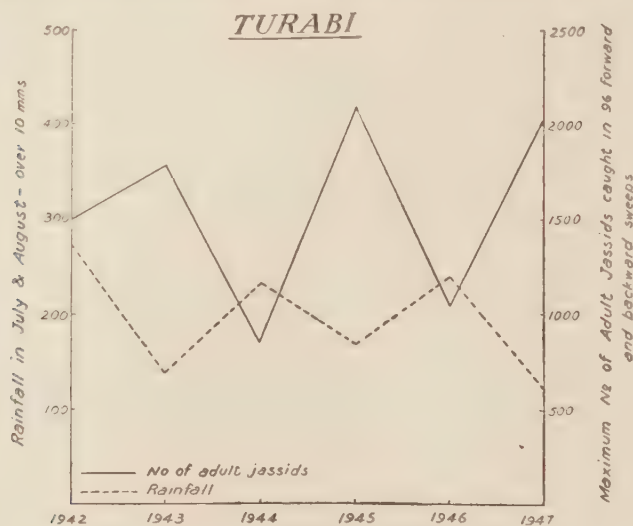


Fig. 2.

This experiment was repeated over an equal area of heavily infested cotton at Turabi (on 18.xi.1947) and the number of nymphs per 100 leaves before and after spraying was 1,424 and 119, respectively—a reduction of 91.6 per cent.

The inference is that rain, if it is to have the same effect as spraying with mud, will only be effective if it is heavy enough to produce mud splashing. The height to which mud will splash was determined by means of a little apparatus consisting of a wooden pole inserted into the ground at the top of which was a short horizontal wooden arm, about 20 cm. in length, attached to it at right angles. A tin cone about 17 cm. in diameter and 16 cm. in length was suspended from this arm with its base directed downwards. A circular cork pad 12.5 cm. in diameter was fitted horizontally inside and kept in position by three short tin straps soldered to its surface. On the lower surface of the cork pad a filter paper of the same diameter could be fixed by small drawing pins (fig. 3).

Fifteen such outfits were set up on flat soil in the Gezira Research Farm. The filter paper in the first was 10 cm. from the soil, each of the others being 10 cm. higher than the preceding one. When it rained the mud was splashed up on the filter papers which were carefully removed and dried. The dust was then swept off with a fine brush and weighed. The results are given in Table II.

It is evident that the mud splash rises little more than 30–40 cm. but the amount of rain is not the only factor involved. It also depends on the velocity with which the drops fall, the size of the rain drops, the interval between each shower and the steepness of the soil slope and its direction.

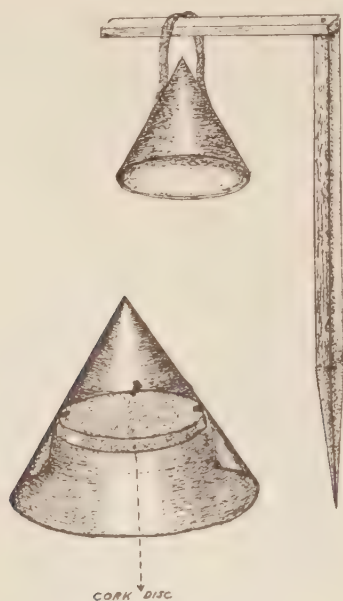


Fig. 3.

TABLE II.

Date	Amount of rain in mm.	Weight of dust in gr. at a height of — cm.						
		10	20	30	40	50	60	70-150
9.viii.1948 ...	27.0	1.25	0.57	0.21	0.02	0.005	Tr	—
16.viii.1948 ...	20.8	2.29	0.33	0.12	0.19	0.005	Tr	—
20.viii.1948 ...	16.1	2.60	0.58	0.42	0.24	0.007	Tr	—
3.ix.1948 ...	8.9	0.36	0.29	0.24	0.05	0.003	Tr	—
7.ix.1948 ...	50.9	2.80	2.04	0.85	0.115	0.04	Tr	—
12.ix.1948 ...	12.9	1.65	0.80	0.19	0.02	Tr	—	—
24.ix.1948 ...	3.7	0.11	0.10	0.01	Tr	—	—	—
29.ix.1948 ...	18.8	1.15	0.50	0.28	0.07	0.03	Tr	—
17.x.1948 ...	6.4	0.24	0.14	0.08	0.05	Tr	—	—

This was shown by examining some plants of *Solanum dubium* grown on the two sides of an irrigation field channel (Abu Ishreen), running from East to West. The number of Jassid nymphs per 100 leaves on the Northern and Southern side of the canal were 140 and 309, respectively, on 2.viii.1948 and 190 and 330 on 4.ix.1948.

The prevailing wind during the rains in the Gezira is usually from South to North so that the rain drops fall almost perpendicular to the Northern side and parallel to the Southern one. It is obvious therefore that the mud splash would be more on the lower side of the leaves of the Northern than the Southern side and this explains why there were more Jassids on one side than the other.

Cover of Vegetation on Soil.

It has been noticed that the cotton growing next to dura (millet) has more Jassids than that growing next to fallow land. Three cotton "numbers" next to dura were

examined in Turabi (North area) on 6.x.1948 and were found to bear an average of 457 nymphs per 100 leaves while the three "numbers" away from dura had an average of 138 nymphs per 100 leaves.

The probable explanation is that dura is sown about two and three weeks earlier than cotton and the soil, when watered, supports a great deal of weed growth on which Jassids live. The spreading leaves of dura protect the soil from mud splash, and as a result of this, the number of Jassids increase and they soon migrate to cotton when it appears.

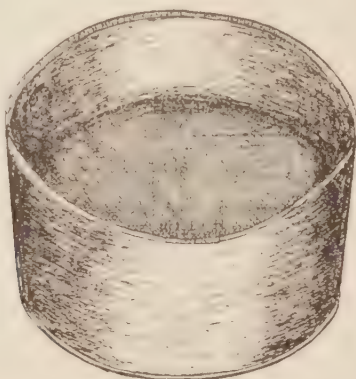


Fig. 4.

It is worthy to note that *S. dubium* growing on fallow land in the summer supports only a small number of nymphs, but when it grows on the banks of the irrigation canals, with a great deal of grass underneath, there are more nymphs. Counts of nymphs per 100 leaves (11th August, 1948) on *S. dubium* growing over grass and also on fallow land was found to be 134 and 5, respectively.

Mechanical Constitution of the Soil.

Samples of surface soils from different parts of the Gezira (North and South) were collected and each sample was placed in a small 2 oz. tobacco tin the bottom of which was replaced by a very fine wire gauze covered by a circular piece of fine muslin (fig. 4). The samples were first dried and weighed, then they were exposed to rain and again dried and weighed. The average percentage of loss in the soil is shown in Table III.

TABLE III.
Percentage Loss.

Date	Amount of rain in mm.	% of loss in Northern Gezira soil	% of loss in Southern Gezira soil
9.ix.1948 ...	50.8	19.6	25.5
13.ix.1948 ...	12.4	7.8	9.2
30.ix.1948 ...	18.5	6.5	8.1

It is clear therefore that the splash rate is greater with soil from the Southern than the Northern Gezira. This coincides with the mechanical analysis of these soils

carried out by the Chemical Section, which gave a clay content of 58 per cent. in those from the Southern Gezira and of 49 per cent. from the Northern Gezira.

The great reduction in the number of Jassids after spraying with mud, suggests that rain might have the same effect, but this required confirmation.

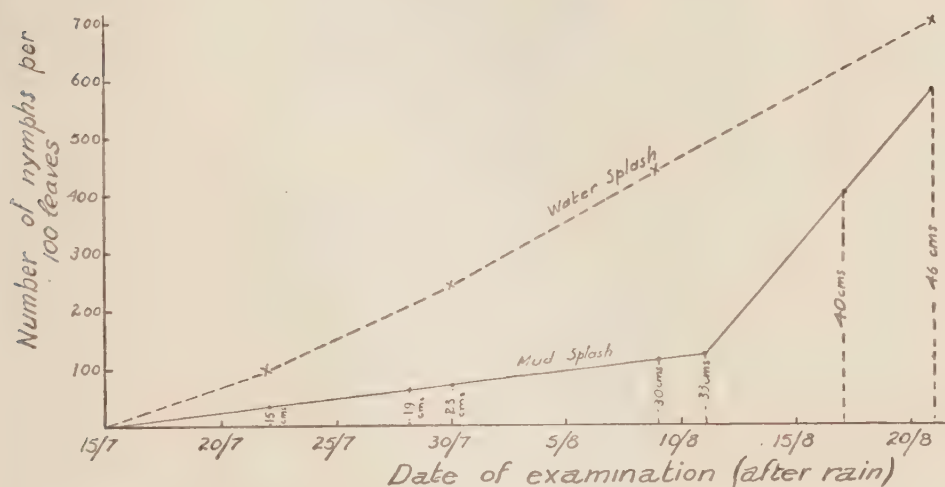


Fig. 5.—The increase in the number of Jassids on water and mud-splashed plots.

Egg-plants (*Solanum melongena* L.) which are a favoured host for cotton Jassids were planted in a plot about the first week in June (the heavy rain season started in July). The plot was divided into two sections: in one, the soil between the plants was covered with broken red bricks so that rain would only produce water splash and not mud whilst, in the other, the soil was left exposed to the direct effect of rain.

Counts of nymphs were taken before the experiment started and the number per 100 leaves was 72 in the first and 80 in the second. After every rainfall the nymphs were again counted. The results plotted in fig. 5 show that:—

(a) The number of Jassids on the egg-plants in the covered plot increased rapidly and hopper burn was frequent and pronounced on the leaves.

(b) In the exposed plot, the number of Jassids increased very slowly for a time after which the increase became sharp and sudden. The height of the plants when this sudden increase commenced was found to be 33 cm.; the plants had grown beyond the range of mud splash.

It might be argued that covering the soil with bricks would produce weak and unhealthy plants leading to heavier Jassid damage. Egg-plants were, therefore, planted and the soil covered with red bricks on the same date as the previous experiment. The plants were sprayed once a fortnight with 0.1 per cent. DDT emulsion to get rid of all Jassids and they developed normally showing no signs of ill health as a result of covering the soil with red bricks.

Jassids on the mud-splashed leaves could be seen plastered on the leaves and embedded in the mud. It is a striking feature that the mud is usually most dense round the veins, especially in the angles between them and the lamina, where the Jassids commonly live (fig. 6). If the leaves are taken after rain, dipped very carefully in a dilute solution of gum and then dried, the mud collected on them can be conveniently examined without falling off.



Fig. 6.

It has been mentioned earlier that, during the dead season (May and June), there is no vegetation in the Gezira except in the gardens of the Inspectors and round the irrigation canals and river bank. There are also some scattered trees, mainly *Acacia*, which harbour Jassids. When the rainy season starts late in June, weeds appear everywhere and Jassids seem to migrate to them from these gardens and trees. The inference from the experiments described above is that rain in July and August kills by mud splash a high proportion of the Jassids on these weeds before they spread to the cotton which is usually sown about the middle of August. The rain that falls in the second half of August also kills a large number of Jassids that have already migrated to the young cotton. September and October rain appears to have little influence as the spreading leaves of the cotton plants protect the soil. A very strong rain storm, however, may produce considerable mud splash despite the height of the plants.

The above evidence suggests that mud splash by heavy rain storms in July and August is probably a limiting factor in Jassid distribution in the Gezira.

The Possibility of Forecasting the Extent of Jassid Attack on Cotton.

It has already been shown (figs. 1 and 2) that there is a relationship between Jassid population on cotton with the amount of rainfall in July and August. This

might be used as a basis for forecasting (fig. 7). The evidence from the data available is that if the amount of rainfall (of showers over 10 mm.) is above 250 mm., the Jassid damage on cotton is negligible, from 250 to 230 mm., the damage is slight but less than 230 mm., the damage is considerable.

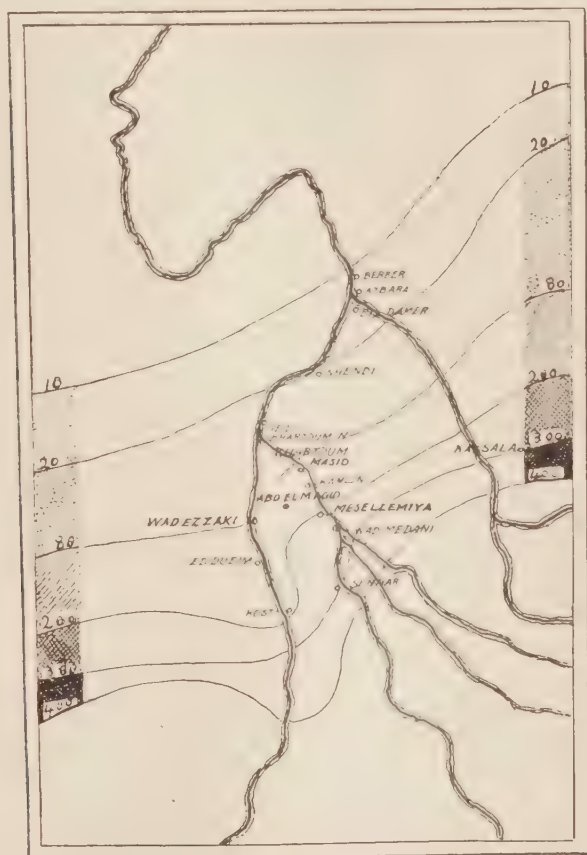


Fig. 7.

It must be pointed out here that, although the amount of rainfall (in July and August of showers over 10 mm.) was the lowest on record in Turabi area in the season of 1948 and it was accordingly expected that the number of Jassids on cotton would be very high, yet it was, on the contrary, very small. The number was actually very high at the beginning of the season but was abruptly reduced by a very violent sand storm on the 19th September which apparently originated in the West of the Middle area and travelled Northward to Turabi in the Northern area.

Table IV gives the number of nymphs per 200 plant holes in the Northern Gezira in 1947 and 1948.

TABLE IV.

1947				1948			
September	Locality	Cotton "Number"	No. of Jassids	September	Locality	Cotton "Number"	No. of Jassids
10th ...	Awoda ...	6	45	9th ...	Hebeika ...	7	535
		2	45			5	71
	Abo Ros ...	10	31	11th...	Azrag ...	17	640
		7	29			33	108
11th ...	Om Hemeir	6	46		Kirsh El Fil	6	61
		8	35			19	314
	Kirsh El Fil	11	146		Egeida ...	7	339
		16	94			23	142
13th ...	Haram Sereiha	8	200		Harig ...	12	91
		5	38			2	223
		36	602	12th...	Abo Wafi ...	17	1,463
		47	262			30	94
14th ...	Badr ...	52	436		Heneina ...	1	298
		63	172			12	211
		10	494		Abo Ros ...	7	513
		16	511			21	183
		15	507	13th...	Shobali ...	1	71
		4	178			20	144
Average 215			3,871	Average 306			5,407

The number of nymphs at the beginning of the 1948 season was therefore 29.7 per cent. more than in the previous year.

Table II shows the number of nymphs per 200 plant holes before and after the sand storm in given localities in the Northern Gezira.

TABLE V.

Before the sand storm			After the sand storm		
September 1948	Locality	No. of Nymphs	September 1948	Locality	No. of Nymphs
18th ...	Abo Ros ...	639	23rd ...	Abo Ros ...	29
19th ...	Shobali ...	199	23rd ...	Shobali ...	44
19th ...	Abo Wafi ...	1,020	23rd ...	Abo Wafi ...	552
19th ...	Heneina ...	565	23rd ...	Heneina ...	155
Average 606			Average 195		

The reduction after the sand storm was 67.8 per cent. The velocity of this sand storm was so great that it stripped the cotton of some of its leaves in the West of the Middle areas and in the Turabi area leaves could be seen torn or scorched by the friction of the sand particles.

It is not known yet whether the effect of such a sand storm on Jassids was due to:—

- (1) The evaporative power of the air.
- (2) The mechanical action of the wind in carrying them to unsuitable places.
- (3) Interfering with the normal activity of the insect.
- (4) Direct injury.

It should be noted that such storms are common in April and May before the rains break, but have never previously been recorded in such strength in September, so that this particular storm must be regarded as most unusual.

Summary.

The Gezira Scheme is divided into three areas according to the incidence of the Cotton Jassid, *Empoasca lybica*.

1. The Northern area of normal abundance.
2. The Central area of occasional abundance.
3. The Southern area of possible abundance.

The causes for this difference in the number of Jassids in the three areas were studied and rainfall in showers over 10 mm. in July and August was found to be the important factor. A definite correlation was found between the number of Jassids on cotton and the amount of this rainfall.

Some experiments were carried out which proved that the effect of rain was due to the splashing of mud from the soil on to the lower side of the leaves. The rain could therefore be effective if it is heavy enough to produce mud splashing.

It was shown by a simple apparatus that mud splashing hardly rises more than 30-40 μ m. /c

The correlation between the number of Jassids and the amount of rainfall in July and August might be used as a basis for forecasting the extent of Jassid attack on cotton every year.

Acknowledgements.

I wish to express my thanks and gratitude to Mr. J. W. Cowland, Mr. R. C. Maxwell Darling, Senior Entomologists, Dr. T. N. Jewitt, Chief Chemist, Mr. H. Ferguson, Plant Physiologist of the Research Farm, and Sayed Effendi El Tayeb, Technical Assistant, for their unfailing help.

References.

- COWLAND, J. W. (1947). The Cotton Jassid (*Empoasca libyca*, Berg.) in the Anglo-Egyptian Sudan, and experiments on its control.—Bull. ent. Res., **38**, pp. 99-115.
- COWLAND, J. W. & EDWARDS, C. J. (1949). Control of *Empoasca lybica* de Berg. on Cotton in the Anglo-Egyptian Sudan.—Bull. ent. Res., **40**, pp. 83-96, 1 pl. 3 figs.
- UVAROV, B. P. (1931). Insects and climate.—Trans. ent. Soc. Lond., **79**, pp. 1-247.
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STUDIES ON BEETLES OF THE FAMILY PTINIDAE.*

III.—A TWO-YEAR STUDY OF THE DISTRIBUTION AND ABUNDANCE OF *PTINUS TECTUS* BOIELD. IN A WAREHOUSE.

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Among the many problems associated with the investigation of populations of insects in warehouses, one of the most pressing is to find adequate methods for determining the distribution and actual numbers of insects present. Until this problem is satisfactorily solved, work on many others is difficult or even impossible. This paper is the outcome of a two-year study of the population of *Ptinus tectus* Boield. in two rooms of a warehouse in High Wycombe, Bucks. Although the data relate to this particular species and situation, both the methods and findings should be of much wider interest and application.

The problem of estimating numbers under warehouse conditions is made more difficult by the fact that many of the insects being inaccessible, their numbers must

*Parts I and II were published in Ent. mon. Mag., 85, pp. 137-139, 189-190. 1949.

be assessed indirectly. Economic requirements raise an aspect of the general problem which is given special attention here, namely, to find methods by which a rapid estimate of the numbers present can be reached in the course of a single inspection. Such methods, if they are to be reliable, must necessarily take account of the habits and behaviour of the species concerned under various conditions and so allow for daily, seasonal and other changes in distribution.

P. tectus was first found in Britain about 50 years ago and since then it has spread rapidly and has become one of the most widespread of general warehouse pests. It will grow and develop on a wide variety of stored foodstuffs. It is confined to temperate areas, having low maximum and optimum temperatures for development and oviposition; it is active at 2 C. and will sometimes lay an egg at 5 C. In the warehouse in Britain it completes two, occasionally three, generations a year. Some adults probably live for more than a year, although the maximum life of a marked beetle in these observations was nine months.

Ptinus is responsible for various types of damage: it consumes the foodstuff, adds excreta, silk and fragments to it, and spoils the fabric of the rooms and sacks it invades, spilling the food in doing so.

Insects need to be very numerous before the direct consumption of foodstuff becomes serious. Rough calculations based upon respiration rates show that about 10,000 *Ptinus* larvae consume only about 1 kilogram of food in completing their development, so it requires about 700,000 to eat one 140 lb. sack of flour.

Nevertheless, *Ptinus* can become so numerous that they destroy the food value of the materials. In one dark damp warehouse in 1946, copra cake was so badly infested that the sacks started falling to pieces. Samples taken from the middle of the sacks were tested after the adults and larvae had been sieved off and were shown to be very inferior to the fresh uninfested food. Much waste is caused by leakage through the bags owing to the insects continually passing in and out between the meshes of the sacking, but *Ptinus* causes much less waste in this way than *Tribolium*.

The extent of the nuisance caused by silk and debris is illustrated by the fact that 80,000 cocoons were left in the food or stuck to the 50 small bags stored in 1946 which contained a total of 75 kg. of food. Frass from all these insects is left in the food together with strands of silk spun by feeding larvae. The sacks on which cocoons are spun are weakened by them and by the emergence of the adult, though the final tearing of the bags is probably as often due to the high humidity, which enables large populations to build up, as to the insects themselves. Wandering fully fed larvae preparing a pupation site can do a considerable amount of damage. They often hollow out a depression to fit their cocoon and are able to eat into wood with ease, attacking good seasoned timber. In this warehouse they made it possible to lift off large pieces of wood from the floor planks.

The Environment and the Population.

These observations were made in two rooms of a warehouse at High Wycombe, Bucks, at weekly intervals from January 1945 to October 1946.

The warehouse was a converted furniture factory in a poor state of repair, and the two rooms used (Nos. 69 and 70) were on the top (fourth) floor. There were some holes right through the floor which was weak everywhere and many upholstery tacks had been knocked into it. Some of the cracks between the boards were very wide. The floor was generally a single board in depth, but in some places was strengthened by a second board underneath. In other places there was an underboard, and the normal floor had been removed.

The outer walls and dividing walls between the rooms were of whitewashed brick, and generally in good condition. Most of the windows were covered by black cloth but enough were left clear to give sufficient light for writing during daylight. The sliding door in Room 69 was opened whenever this room was examined, giving good light over the whole of the room. A few broken window panes let in rain and winds.

The roof was slate with a wooden lining but was leaky and a leak in the S.E. corner of Room 70 was so bad that a tarpaulin was placed over the sacks of flour to keep them dry. Three wooden rectangular pillars supporting the roof were evenly spaced across each room.

When observations began the rooms were empty. The flour which had previously been stored had just been removed, and the sweepings placed in a piece of sacking and left in Room 69. The sweepings contained a large number of *Ptinid* larvae and some adults of *Ptinus tectus* and *Niptus hololeucus* (Fald.).

A recording thermohygrograph was placed on the beam which ran lengthways along the middle of each room. The empty rooms were examined during January and February and no insects were found on the walls, floor or posts except in a bag of sweepings. The floor cracks were probed with a blunt needle but no insects were found.

Sacks of Canadian vitamin-fortified white flour arrived at the warehouse about 20th February 1945 and were stowed in the two rooms in a somewhat irregular fashion. Gaps were left where the floor was especially weak or where the roof above leaked badly or where paths to the crane and loading door were needed. About one-quarter of the floor space adjacent to the dividing wall in each room was left clear.

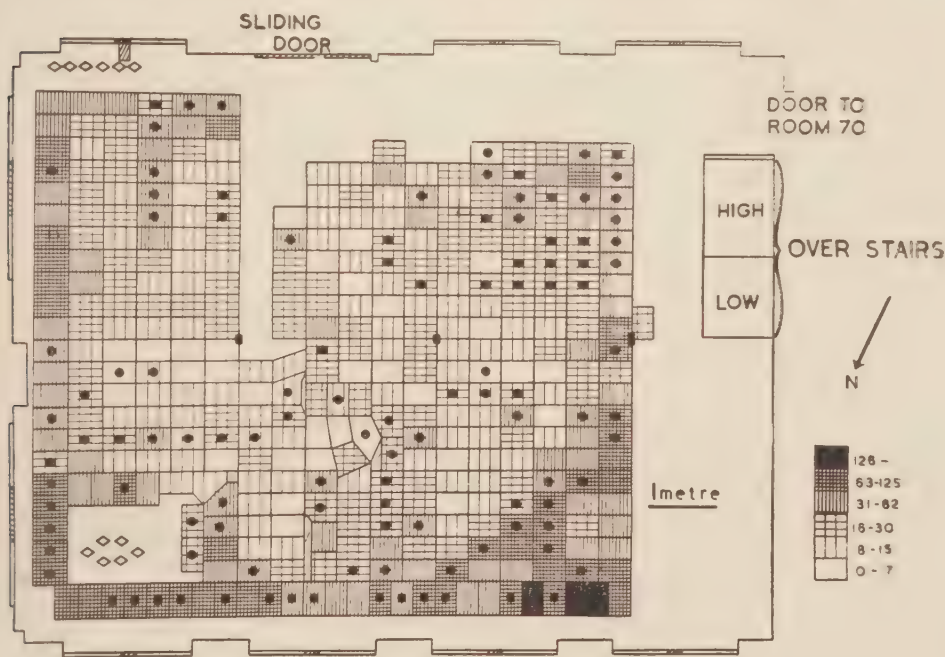


Fig. 1.—Plan of Room 69 showing the arrangement of the 366 sacks. Cotton sacks are shown by a spot ● and the position of oviposition traps by a diamond <>. The shading represents the total numbers of adult *P. tectus* found on all visits. The three rectangular spots represent posts supporting the roof. Double lines around sides of the room represent windows and single lines brick walls.

Most of the sacks were stood upright and, since the floor was too weak for a heavier load, stacked only one tier deep. This is an unusual method of stacking but it made possible a number of successive examinations of every sack of flour. The results obtained are not necessarily applicable to the more normal methods of stacking, but it seems from other observations that they do in fact give a good picture of the normal events.

Figs. 1 and 2 illustrate the plans of Rooms 69 and 70, respectively, together with a diagrammatic representation of the position of sacks at the beginning of these observations.

In both rooms the supporting posts interfered with the regularity of stacking, especially after one or two examinations. Originally there was a path a foot or so wide round the stacks, but as continual examination loosened the tightness of packing, this space was gradually reduced. From time to time also, the warehouse staff moved odd bags that had become damp on account of leaks or broken windows. Numbers of beetles were soon to be found on and around the sacks. They laid eggs into the flour through the sacking, so that a population developed inside the sacks as well as in cracks in the floor.

This flour was removed from the warehouse at intervals from October to December 1945. The floors were swept early in December and an insecticidal dust spread over them. Subsequent examination in December and January of areas which were first vacuum cleaned showed that there were large numbers of free larvae and cocoons containing larvae or pupae, still present in cracks in the floors. Later observations showed there must also have been a large population of beetles in the fabric of the building.



Fig. 2.—Plan of Room 70 showing the arrangement of 401 upright sacks and 24 flat sacks. Other symbols as for figure 1. The line A B divides the long section from the "square" section mentioned on page 386.

On 17th January 1946, 25 small jute bags (subsequently referred to as "experimental bags" or simply as "bags") made from sacking were each filled with 1.5 kg. of wheatfeed (fine middlings) and placed in a square group in Room 69. A second

similar batch was put down nearby on 29th January 1946. Five bags from one batch were removed and replaced on 14th May 1946, one from each batch was taken away and replaced on 10th July 1946 and the whole of one batch was moved at the beginning of October. The contents of the other set were not examined.

Although a large number of insects that attack stored products could be found in the warehouse, few were found in the rooms here described and no species other than *P. tectus* was at all common. The commonest other Ptinid was *Niptus hololeucus* of which 216 specimens were collected and removed. These emerged mainly between August and October when they comprised up to 2 per cent. of the Ptinid population. Ten specimens of *Ptinus fur* (L.) were collected in October and November. *P. pusillus* Sturm, *P. sexpunctatus* Panz. and *Trigonogenius globulus* Sol. were found once or twice. *Attagenus pellio* (L.) was common in Room 70 but not in Room 69, 14 adults and 140 larvae being recorded. The numbers of specimens of other species seen were :—18 *Cryptophagus* spp., 6 *Enicmus minutus* (L.), 2 *Tenebroides mauritanicus* (L.), 22 *Lycocoris campestris* (F.), 5 *Ephestia kühniella* Zell., 9 *Hofmannophila pseudospretella* (Stainton), 7 *Tinea pallescentella* Stainton, and 1 *T. ditella* Pierce & Metcalfe.

Few Ptinid parasites were found. A few Hymenoptera were seen and collected on 2nd October, 1945, and were identified by Dr. O. W. Richards as :—*Blacus humilis* (Nees) (parasites of Lathridiids), *Lariophagus distinguendus* (Foerst.) (beetle parasite), *Stenomalus muscarum* (L.) (hibernating) and 1 *Hemitrichus rufipes* Thoms., a Ptinid parasite. The common parasite of *P. tectus*, *Dimachus discolor* (Walker), was not recorded.

On the small bags placed in Room 69 in 1946, *Cryptophagus*, *Niptus*, and *Hofmannophila* were the commonest minor species. *Cryptophagus* was commonest from February to May and after that was not often found. *Niptus* again showed a peak of emergence during late August, though many fewer were found this year partly because they had been collected for laboratory culture the previous year. *Hofmannophila* was most abundant during July. The total number of each species found on and around the bags in all examinations were :—

Ptinus tectus 42,295 adults

P. fur 11 adults

P. pusillus 1 adult

Cryptophagus spp. 120 adults

Attagenus pellio 1 adult and 1 larva

Laemophloeus sp. 1 adult

Tinea pallescentella 1 adult

Blattella germanica (L.) 1 adult

Niptus hololeucus 52 adults (most removed)

P. sexpunctatus 3 adults

Trigonogenius globulus 2 adults

Enicmus minutus 5 adults

Oryzaephilus surinamensis (L.) 3 adults

Hofmannophila pseudospretella 49 adults and 1 larva

Scenopinus fenestralis (L.) 3 larvae

Lepisma saccharina L. 1 adult

Hofmannophila was breeding in many parts of the building and in the autumn larvae could be found in most rooms.

Estimation of the Numbers present.

Beetles on the sacks and bags.

Six examinations were made during 1945 of the outside of the 266 sacks in Room 69 and five of the 401 sacks in Room 70. In addition nine very thorough examinations of the sacks and the floor around them were made on samples of about 50 sacks, and of the last 93 sacks of Room 69, as they were being removed.

While it was impossible to count the total number of adult *Ptinus* in the rooms, it was desirable to have a reliable estimate to record the population changes occurring. Therefore the laborious process of counting and recording the *Ptinus* present on each

sack individually was undertaken. The parts of the sacks examined were the tops and all of the sides except the bottom six inches, *i.e.* the parts of the central sacks of the bulk which could be examined without an excessive amount of labour. Care was taken to avoid examining a greater area on outer sacks. Although adults were often visible on the floor or at the base of the sacks, they were ignored in these counts.

Counts were started at the outside edge of the bulk. During examination of a sack, it was pulled away from the bulk or laid down on its side and then the neighbouring sack on which the first had been leaning was examined. Rows in which one sack leans on its neighbour will in future discussion be called "natural rows". At the outside edge of natural rows counting was easy, but the space available became reduced towards the middle of the rows, so that here it was nearly always necessary to lift out a sack after it had been examined in order to make a space to move its neighbours for examination.

As the insects congregated mainly where sacks were only lightly pressed together few, if any, insects were knocked off the sacks so shifted. After a sack had been moved, the neighbouring sacks could be examined by rocking them on their bases.

A casual examination of the sacks at the edge of the bulk a few days after the flour arrived showed that there were insects on one or two of them. The total number of adult *Ptinus* found in subsequent examinations is shown in fig. 3.

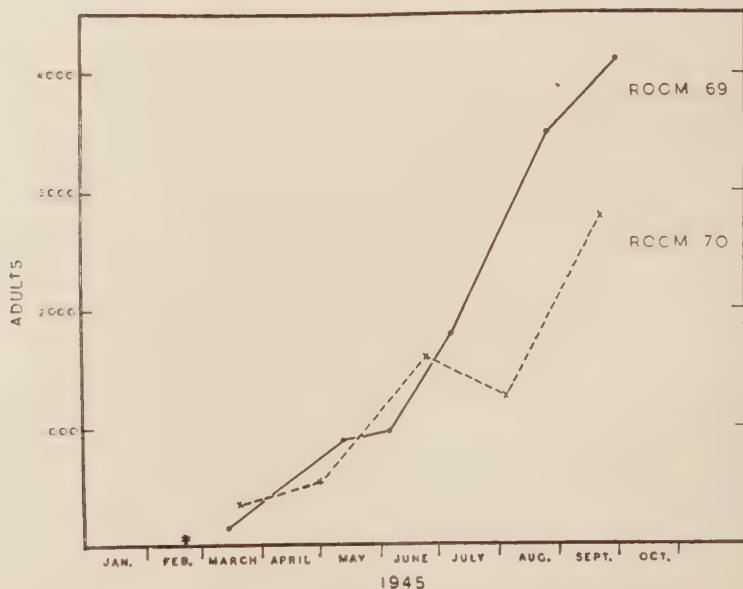


Fig. 3.—Numbers of adult *P. tectus* found on the sides and tops of the sacks of flour during 11 examinations during 1945. The star indicates the date on which the flour arrived.

Since only a part of the population (*i.e.* that at the top and sides of each sack) was counted, the estimates of numbers fluctuate considerably according to the distribution of the beetles between the various sites. This distribution is affected by weather conditions and as a result small numbers of adult *Ptinus* were found in cold or in very bright weather.

Conditions in Room 69 did not vary quickly, but Room 70 was liable to obvious day to day variations especially in the amount of sunshine. This room was quite unsheltered on the windward side where there were a number of broken windows. Bright sunshine, wind and low humidity probably explain the apparent drop in population in July. Even in Room 69 by 18th September it appeared that the rate of increase had slowed markedly. It may be that falling temperatures had induced some adults to leave the sacks to hide in cracks as well as reducing the speed of development.

The foregoing data relate only to the numbers of adults found on the top and sides of bags, but on ten occasions in 1945 a group of about 50 sacks was chosen for a more complete examination which included the base of the sack and the floor nearby.

The data show that about one-third of the total adult population of the outside of a sack is normally at the base or on the floor and consequently was omitted from normal counts, but this estimate is a rough one since the areas in which floor examinations were made were not entirely representative.

It was thought that some adults might be missed when the layer of flour on the floor was thick. To check this, a comparison was made on one occasion between the number of adult *Ptinus* observed by visual inspection of an area and the number collected from the same area by vacuum cleaner. The comparison was made on 11th September, 1945, on 17 sacks, and the floor on which they stood was in a region where flour spillage was especially thick.

Visual examination showed 35 living adults and a number of dead which were not recorded. Vacuum cleaning produced 53 living and 51 dead adults, the collection being divided into 34 samples (one from each bag and one from the floor beneath). This method of examination of the vacuum collection made it possible to show that at least seven of the 35 living adults originally observed were killed by the vacuum cleaner. Thus the original population consisted of at least 60 living adults and not more than 44 dead, and in this area, where the conditions for visual examination were extremely poor, over 40 per cent. of the adults were missed. Normally, the conditions were much better than here so that the numbers were probably subject to a much smaller error.

After the flour had been removed and the small bags of wheatfeed had been put down in Room 69 in January 1946, the insects found on these two batches of bags were recorded weekly, the numbers present on the tops, fronts, backs, bottoms, and floor under bags being recorded separately for each bag.

With a record for nearly every week these figures were more sensitive to weather change than those obtained in 1945. The totals for the two sets of bags followed very similar trends (fig. 4). No insects were seen during the initial cold spell in January but thereafter the numbers found increased slowly, fluctuating slightly until early June with slight peaks which show some correspondence with warm spells. From this time until the end of September the increase was very rapid, reaching a total of 5,000. During this period there were two abrupt falls which coincide with falls of temperature, and there was a similar fall in the autumn.

These figures show that the adults frequently leave the stored foodstuff and disappear into the fabric of the warehouse, especially when conditions are unfavourable, so that the number found by an examination of the sacks cannot be simply related to the number actually present.

Although the numbers observed fluctuated, the curve of actual population increase would probably be smooth if allowance were made for the effect of climatic changes. Admittedly there is a sudden rise in June for both sets of bags (fig. 4), but this is probably caused by the emergence of adults produced from 750 early second-instar

larvae which were liberated on one set of the bags in January. This was done in order to investigate the normal period of development of *Plinus* in the warehouse; parallel populations set up in jam jars at the same time also reached maturity in June. There is no likelihood that any substantial number of those present on the bags or on the floor nearby escaped detection at any examination.

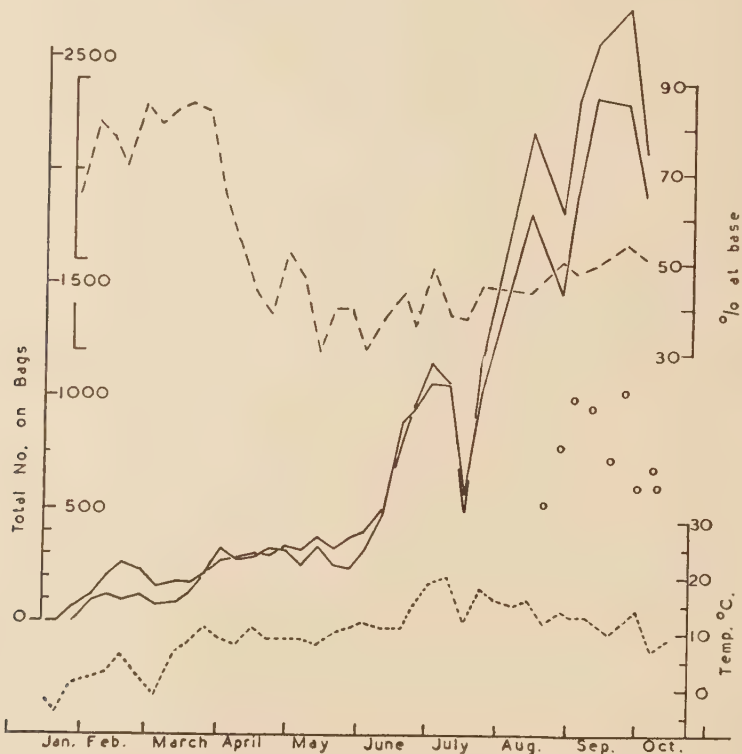


Fig. 4.—The unbroken lines are population curves showing the change in numbers of *P. tectus* adults in each experimental bag-area during 1946. The bottom curve (broken line) represents minimum temperatures during the 24 hours before each examination. The top curve (broken line) is the percentage of adults found at the base of experimental bags at each examination. The number of adults found under oviposition traps is shown by circles on the right.

The regular counts made on the experimental bags during 1946 provide a fairly complete series of figures for the proportion of *Plinus* occurring at the bases of bags, especially since there was insufficient spillage to bury or hide adults. The two areas of bags gave consistently similar results, the mean percentage of insects at the base being plotted in fig. 4. The figures show two marked phases. Up to the end of March a very high percentage of adults was found at the base of bags, the percentage then fell rapidly and oscillated somewhere about 40 per cent. rising somewhat during August and September.

To summarise, there were a few hundred adult *Plinus* on the sacks of flour in each room early in 1945, the numbers increasing to 4,000 in Room 69 by October and to nearly 3,000 in Room 70 by the end of September. Each of these totals should be increased by about one-half to include the beetles on the bottoms of sacks giving values of 6,000 and 4,500 respectively. In 1946, the increase in the total numbers on

a much smaller bulk of uninfested wheatfeed in small bags put into Room 69 in January was broadly similar, and a total of 5,000 on all parts of the bags was reached by the end of September (*cf.* figs. 3 and 4).

Beetles in cracks in the floor.

Attempts were made in 1945 to find adult beetles outside the boundaries of the flour stack. It was unusual to find any, although a few wandering larvae were seen from June onwards and as soon as the floor became covered with flour dust, tracks were obvious, usually beginning and ending in the bulk. The cracks in the floor around the base of the walls were probed as deeply as possible and this part of the floor was vacuum cleaned, but no adults were found there. Adults were never seen on the walls or posts although they are normally obvious there in darker warehouses, but a small number could occasionally be found on the cross-beam supporting the roof.

After the flour had been removed, the warehousemen swept the floors and spread a damp and apparently ineffective insecticidal dust over the floors. Whether or not this affected insects on the floor, it certainly did not reach those in the cracks. In Room 70 the tarpaulin was spread on the floor under the leaky roof and it was soon noticed (in December) that adults were quite numerous under it, especially at the edges and elsewhere where the stitching of hems provided a suitable crevice between the floor and the tarpaulin. On one occasion all these adults were removed but in a fortnight these had been replaced by many others.

During December and January it was possible to find an occasional adult in the floor cracks, but there were very few compared with the number which eventually appeared on the small bags. In October 1946 control measures were started and the floor of Room 69 was swept prior to spraying. Two treatments were used and a sticky band was put down longitudinally along the centre of the room and around the bases of the posts. Another sticky band was put down at right angles to this right across the room, cutting off the door leading to Room 70 and two pieces of floor which received no treatment. About a fortnight later, large numbers of *Ptinus* were noticed stuck to the edge of the transverse sticky band adjacent to the untreated areas of floor. It seems likely that the great majority had been hiding in the cracks at the foot of the wall. This is certainly true near the stairs platform where, although the sticky band was only an inch from the wood partition, very large numbers were trapped.

An attempt to estimate the numbers hiding in the fabric of the empty rooms in 1946 was made by marking insects and setting traps. The proportion of marked and unmarked beetles in the traps and the number of marked beetles released can be used to estimate the population, provided enough time is given between the release and capture of marked specimens to allow them to find the hiding places used by the resident population.

The traps used were pieces of newspaper on which was placed some foodstuff. The beetles collected under the paper instead of going to the more usual hiding places at dawn. The method of marking is described later (p. 392). Marked beetles, about 500 altogether, were released several times during 1946. The numbers caught in traps each week were small, so the estimates obtained are only rough. Over the period July to September 1946 only one in 300 of the beetles found in traps were marked, that is about 2 per cent. of the marked beetles released. The result obtained by multiplying the numbers of beetles trapped by 50 or the number of marked specimens released by 300 is about 150,000.

It is of interest to note (circles on fig. 4) that the number of adults found under traps during this period remained consistently about one-fifth of the number present on the bags.

Larvae in cracks in the floor.

Larvae and cocoons were found quite easily in the floor-cracks during November and December 1945 by probing with pointed needles. An area approximately 5 feet square at the west end of Room 69 was swept and vacuum cleaned, and the entire length of eight cracks in the area examined with a needle. The numbers of cocoons and large larvae found in each of the eight cracks were 5, 10, 8, 20, 16, 20, 17 and 12 (-1 ad.) (Mean 13.5, Standard Error = 2), equivalent to a total population of about 7,000. In a similar area near the middle of the E. wall there were 2, 31, 15, 38 (-2 ad.), 16, 19, 36 (-1 ad.) and 14 (Mean 21.4, Standard Error = 4.4), equivalent to a total population of about 11,000. Both these areas lay within the space formerly covered by sacks but did not correspond with areas of the previous heaviest infestation. Thus it appears that between 7,000 and 11,000 larvae would have been left behind even if the whole room had been vacuum cleaned. Twelve cracks, chosen at random in Room 70 in February 1946, after vacuum cleaning gave 33, 15, 6, 9, 31, 8, 24, 13, 43, 12, 12, 7 (Mean 17.75, Standard Error = 3.5) equivalent to a total population of approximately 10,000.

An estimate of the population removed by the vacuum cleaning was obtained on 6th November 1945, ten days after the unloading of this room was started. In the S.W. corner eight areas each 3 ft. square were vacuum cleaned and the material picked up was retained and examined. The catches in these areas were 8 (-2 ad.), 19 ($+1$ ad.), 7, 11, 17, 31 ($+7$ ad.), 5, 3, a total of 99 larvae and 10 adults. This is equivalent to 7,000 larvae for the whole loaded part of the room but is possibly an overestimate since this was a heavily infested area. The living adults found were equivalent to only 700 for the room, but 42 (equivalent to 3,000) dead adults were found. It is likely that many others were taken away with the sacks or had migrated to those remaining when this treatment was carried out.

Briefly then, it was estimated from the examination of sample areas that there were between 7,000 and 11,000 larvae in cracks in the floor of Room 69 in November-December 1945 and 10,000 in Room 70 in February 1946. In addition there were numbers of larvae in the loose material removable from the floors by vacuum cleaning; these were estimated at 7,000 for Room 70 in November 1945.

Larvae inside the sacks of flour.

So far insects inside sacks have not been considered. Most of the real damage must of course be done by the insects which occur in the flour itself, so it becomes important to know what proportion of the population actually occurs inside the sacks.

It appears that the adult beetles are unwilling to enter sacks full of flour so long as there is sufficient food available to them outside the sacks. Some adults, however, do enter the sacks at the tops (usually through the tied or sewn neck) probably because at the top there is some free surface on which they can move freely. Nevertheless it is a common observation, especially in late summer, that there are numbers of adults inside sacks with just the heads and antennae projecting; they presumably emerge from this situation under suitable conditions. These adults are sitting in the larval cocoons, the majority of which are spun against the sacking, and are most likely to have developed inside the sacks.

It is important to ascertain the number of larvae in the flour, especially as this is the main feeding stage, but owing to the difficulty of obtaining satisfactory samples from the bags, this problem has not been solved. Samples of the contents of the sacks taken with a (100 cc.) grain sampling spear (Oxley & Henderson, 1944) did not produce a single larva, probably because by this method the centre of the sacks is sampled. A surface sampling method was tried later; a sack was selected, laid flat and an X-shaped cut made in the upper side. A flat metal plate was then used to skim off an area of flour one foot square. Depths of approximately $\frac{1}{2}$ in. and

2 ins. were obtained easily and were sieved immediately. Larvae were not found by this method except when signs were visible on the outside of the bag at the particular spot. The sign of a larva near the surface of a sack is an exudation of flour which on a horizontal surface forms a conical heap. As might be expected the larvae were most abundant inside the sacking beneath those places where adults congregate, i.e. at the limits of areas where sacks are tightly apposed to each other or to other objects. Larvae also occurred under exposed surfaces of horizontal sacks on which adults were not commonly found.

It is probable that most larvae inside the sacks are introduced as eggs by the adults laying through the sacking and that feeding occurs near the sacking. Thus no conclusion can be drawn regarding the numbers of larvae inside the sacks of flour.

Adults and larvae inside the bags of wheatfeed.

The small jute bags stored during 1946 were examined thoroughly inside and out for adults, larvae and cocoons. On 14th May 1946, five of the bags put in the warehouse on 29th January 1946 (105 days previously) were removed. Four were of a rather looser material than the rest, but one was made of good material. All adults and larvae were brushed from the outside of the bags in the warehouse. There were no cocoons on the bags, either outside or in. The wheatfeed contents were sieved through a 30-mesh sieve to remove the adults and largest larvae; smaller larvae and eggs were bred out and the numbers added to those obtained by sieving. In this way the total *Ptinus* present in these bags on 14th May was estimated to be:—

Good bag	...	0	live adults	...	19	dead adults	...	167	larvae
		2	"	"	17	"	"	221	"
Poorer bags	...	2	"	"	41	"	"	162	"
		1	"	"	23	"	"	227	"
		2	"	"	15	"	"	138	"
Total	...	7	"	"	115	"	"	915	"

The wheatfeed used had been fumigated, since previously it had been infested by *P. tectus*. The dead insects had not been removed, and these account mainly, if not entirely, for the dead adults recorded. No adults could yet have bred through from eggs laid, so the seven living adults must have found a way through the loose mesh of the poorer quality jute. An average of 180 eggs had been laid in each bag in 105 days.

On 10th July one bag from each batch was replaced; 113 and 82 cocoons were counted on the outside of these bags. All the bags of one batch were finally removed four or five at a time between 1st and 10th October 1946. The insects found in the contents of 20 of these bags at this final examination are shown in Table I and a summary of all the results in Table II.

In nine months about 1,500 insects developed from eggs laid in the food in each bag (=75,000 in the total of 50 bags) or about one insect per gram of food. If 1,500 eggs had been laid in each of the large sacks previously stored this would represent ten insects per pound of flour. If 75,000 eggs had been laid in Room 69 in 1945 this would have represented ten insects per seven pounds of flour. These estimates ignore the live adults found on the outside of the bags, a number approximately one-half of those inside, and the adults present in the fabric of the buildings.

Synopsis of data.

The above data are too incomplete to give a true picture of the total number of *Ptinus* actually present in the rooms. In particular there is no quantitative estimate of the numbers of larvae inside the sacks of flour in 1945, and the estimate of 150,000

TABLE I.

Number of insects found in 20 of the experimental bags in October 1946.

Adults		Larvae	Total <i>Ptinus</i>
Dead	Alive		
25	154 + 5A	2,836	3,015
124	287	1,312	1,723
48	259	854 + B	1,161
162	234	1,132 + B	1,528
54	198 + C + A	1,085	1,338
54	453 + A	1,025	1,533
62	168	1,302	1,532
20	181	1,638	1,839
240	168	1,220	1,628
72	285	1,607	1,964
106	295	999	1,400
105	180	1,546 + B	1,831
98	167	1,173	1,438
62	214	1,135	1,411
83	264	749	1,096
29	188	1,450	1,667
40	251	759	1,050
7	298	904	1,209
65	300	1,081	1,446
340	145	2,161 + B	1,701
Total ...	1,796	4,689	25,023
Mean per bag ...	90	234	1,251
			31,508
			1,575

A = *Ptinus fur*B = *Hofmannophila*C = *Cryptophagus* spp.

TABLE II.

Mean number of *Ptinus* in bags in 1946.

Date	Storage period, days	No. of bags examined	Mean No. living <i>Ptinus</i> per bag		Dead adults
			Adults	Larvae or pupae	
14.v ...	105	5	1.4	183	23
10.vii ...	162	2	15.0	1,243	64.5
10.x ...	267	20	234	1,251	90

adults in the fabric of Room 69 in the summer of 1946 is extremely rough and liable to a large error.

The figures for the numbers of beetles on the sacks of flour should be increased by about 50 per cent. to allow for the proportion of beetles at the bases of the sacks where they were not counted. When this is done it can be stated that in Room 70 there were about 500 beetles on the sacks in March 1945 increasing to about 4,200 by September. In the following November there were about 7,000 larvae in loose material on the floor of the emptied room and about 10,000 in cracks in the floor.

In Room 69 there were nearly 400 beetles on the sacks in March 1945, increasing to about 6,200 by September. In late November there were 7,000–11,000 larvae in cracks in the floor. In February 1946 there were about 300 beetles on the small bags of wheatfeed put down the previous month. These had increased to 700 by May (see fig. 4) and by this time there were already 180 larvae and eggs inside each bag.

By the end of September there were about 5,000 adults on the bags, 12,000 adults and 63,000 eggs, larvae and pupae inside the bags.

The Changes in Numbers and Their Significance.

In Room 69 the number of adult beetles visible on the outside of the sacks increased 16 times during the 200 days from March to October. A further slight increase followed in November but the population was apparently decreasing just before the flour was finally removed. When small numbers of sacks were examined thoroughly, about a third of the adults were found to be on the base of the sacks or in the flour. At least 6,000 adults were present in this room when the flour was about to be removed. Some of these would have been removed with the flour and many killed by the handling, but some would be left behind and it is unlikely that they would have remained on the open floor to be removed by sweeping. All stages of *Ptinus*, except eggs and the younger first-stage larvae, have been found alive in winter and spring in cracks and other sheltered places in the warehouse.

If all stages survive the winter in approximately equal numbers, then a gradual increase in numbers of adults would be expected during the spring and the increase would slowly accelerate as development speeded up with the rising temperature. If, however, the stages surviving the winter were restricted to a single instar then the population increases would tend to occur in jumps corresponding roughly with the emergence of succeeding generations.

During three weeks of cold weather in January 1946, no insects were observed on bags containing clean food but afterwards the visible adult population increased gradually to reach nearly 6,000 in October. The maximum rate of increase was in July and August. Examination of the contents of the bags in October showed that these 50 small bags contained about 11,000 adults, 60,000 younger stages, and 5,000 dead adults. The figure of 150,000 adults for the room, obtained by using marked adults, implies that most of the adults live in the fabric of the building. In the small quantity of food used in the oviposition traps, 13,000 eggs were laid during 1946. The total number of eggs known to have been laid by the endemic population and by its offshoots in the bags of wheatfeed was 11,000 + 60,000 + 5,000 + 13,000 = 89,000. These were laid within a period of 250 days, i.e. at a rate of just over 300 a day and with a probable maximum of about 1,000 a day. This could be achieved easily by 10,000 adult beetles and even then the egg laying rate would be much below the normal laboratory result. This suggests that the estimate of 150,000 adults is excessive, but on the other hand the low yield of eggs could be a result of the shortage of water. *Ptinus* larvae seem to be very free of parasitic insects and microorganisms, and death is likely to have been caused only by accident or overcrowding. The normal span of adult life is about six months.

All stages are likely to overwinter, with the exception of eggs and very young larvae. There may be two generations a year with a slight possibility of a third in very favourable circumstances. Overwintering larvae probably all reach adult stage by the end of June, and in early July adults of the next generation are emerging. The enormous infestations which are occasionally noticed probably build up when a moderately big population overwinters and then remains in conditions of a humidity of at least 80 per cent. R.H.

Spatial Distribution and Factors which determine it.

Spatial distribution in stacks of flour.

Figs. 1 and 2 illustrate the distribution in Rooms 69 and 70 of the totals of the numbers found on all visits. Clearly the distribution of the insects is non-random

since they are aggregated towards the outside edge of the bulk of sacks. The average density of insects on sacks in the outer edges of the stack in each room is about twice the mean density for the whole room.

It is necessary to point out that the allocation of the insects to sacks was somewhat arbitrary ; many of them were really found between two sacks but the records were made according to which of the sacks they happened to cling.

It may be assumed that on the average half the insects between sacks were allocated to each sack. Thus it would be expected that sacks at the end of natural rows would have smaller totals than their neighbours in the row. Of the 17 natural rows in Room 69, ten of the end sacks are markedly less infested than their immediate neighbours and only two are markedly more infested than their neighbours.

Although *Plinus* were most commonly found on outside sacks, they were hardly ever found on the exposed parts of those sacks. Thus the single sack outside the W. edge of the main bulk in Room 69 bore few insects, but this sack, and an adjacent support post, provided a suitable site for *Plinus* where they touched the sacks of the outside W. row, and so increased the numbers in this position.

The majority of specimens recorded were found where the larger faces of sacks were opposed, i.e. between two sacks in a natural row. Some did occur at the sides of sacks where two rows were pushed close together but this was much less common.

Where an outside row lay at right angles to a series of natural rows and supported them, a region of high density resulted because in addition to specimens accumulating between the sacks of the outside row, *Plinus* also congregated at the sides of these sacks where the weight of the other sacks pressed. This caused a concentration on these latter sacks as is shown in both rooms (70, S.W. corner ; 69, N. edge ; figs. 1 and 2). Local increases may result from other special features, like the extra outside sack referred to above, or the free faces of the sacks lining the space in Room 69, which were comparable to those of outside rows.

In all natural rows, including outside rows, there was a tendency to smaller densities on the middle sacks (figs. 1 and 2).

Frequently a sack which had been recently wetted was more heavily infested than its neighbours and dampness probably contributes to the high density in the N.W. corner of Room 69.

These conclusions are drawn from a consideration of all the visits paid to the rooms ; the individual records give similar results but there are, of course, exceptions. Fuller consideration will be given later to the results of individual examinations.

Distribution about bags of wheatfeed.

A similar general result was obtained for the two batches of small bags put down in 1946, the distribution of insects being more even but still non-random. Although there were only five bags in a " natural " row there was still a definite predominance of insects at the ends (fig. 5 a). The outer natural rows, however, had smaller totals than their immediate neighbours. These results suggest that the adverse effect of light extends further into the sack than the width of these small bags.

The distribution of insects at floor level was somewhat different, the highest numbers being at the centre of the area. This is illustrated in fig. 5 b which is based on the total numbers found on the bases of the bags and on the floor beneath.

The distribution of adults on the floor and bottoms was certainly related to the topography of the floor. Low numbers were obtained when a bag rested flat and conformably on the floor. Large numbers congregated where the sack was wrinkled or in floor depressions especially around the heads of the numerous upholstery tacks. During periods when the bags were carefully placed on the same floor spot, high or

low numbers were consistently found under a particular bag. When the positions were changed slightly, this correspondence ceased. Fig. 5c compares the floor and bottom total for batch A over the first 11 visits with the totals for the next 11 visits. The slight shift of positions made on the 11th visit removed the obviously favourable spot which had previously held one-sixth of the floor adults, and improved markedly the comparatively unattractive zone.

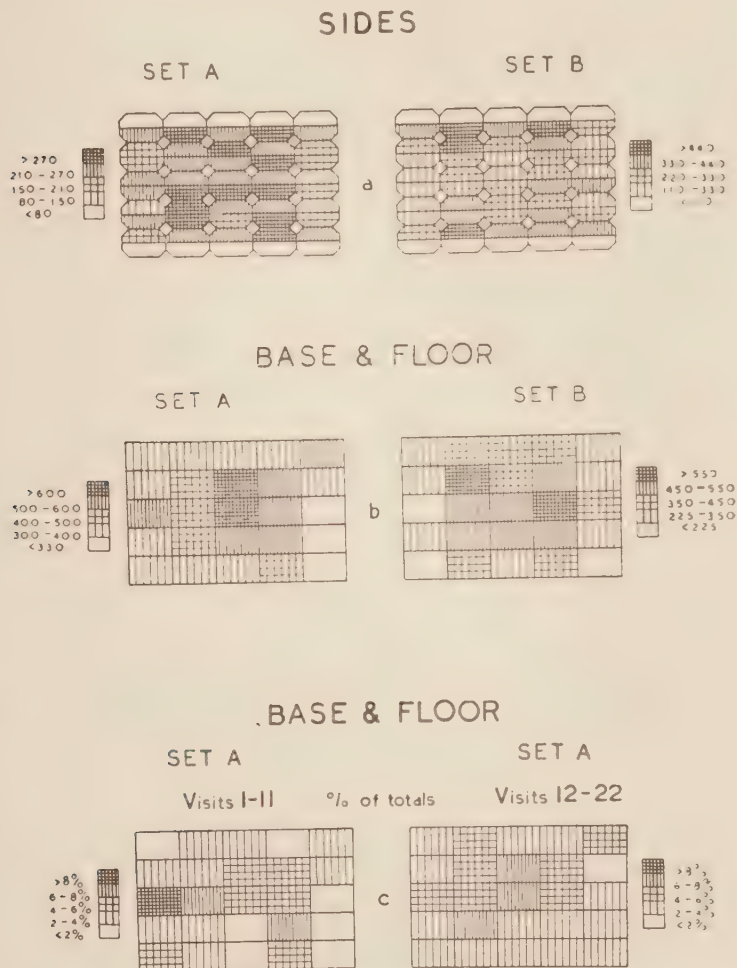


Fig. 5. —Diagrams showing the distribution of adult *P. tectus* on the experimental bags. (a) Total for all examinations on the front and back of each bag. (b) Total for all examinations at the base and on the floor beneath each bag. (c) Percentage of total (for all bags) at the base of each bag and on the floor, showing the effect of a slight change in the position of the batch.

Changes in distribution in a stack of flour.

The data for Room 69 show only very minor changes in distribution from visit to visit, a fact which may be correlated with the relatively equable climatic conditions in that room and accordingly the data are omitted. Room 70, however, was subject

to more violent fluctuation of climatic conditions and correspondingly the data for spatial distribution show substantial changes from visit to visit.

It is convenient to divide Room 70 for this purpose into two sections, a " long " section and a " square " section, along the east to west line A-B (fig. 2) and to consider the data from these two sections separately. First, however, it is worth comparing the total numbers from these two sections visit by visit, viz. :—

	20.iii	1.v	19.vi	31.viii	18.ix
Square section (256 sacks) ...	160	283	667	673	1,365
Long section (144 sacks) ...	187	236	884	558	1,404
Square/long	0.86	1.20	0.75	1.21	0.97

The numbers in both sections follow a similar trend but the variations are more violent in the long section.

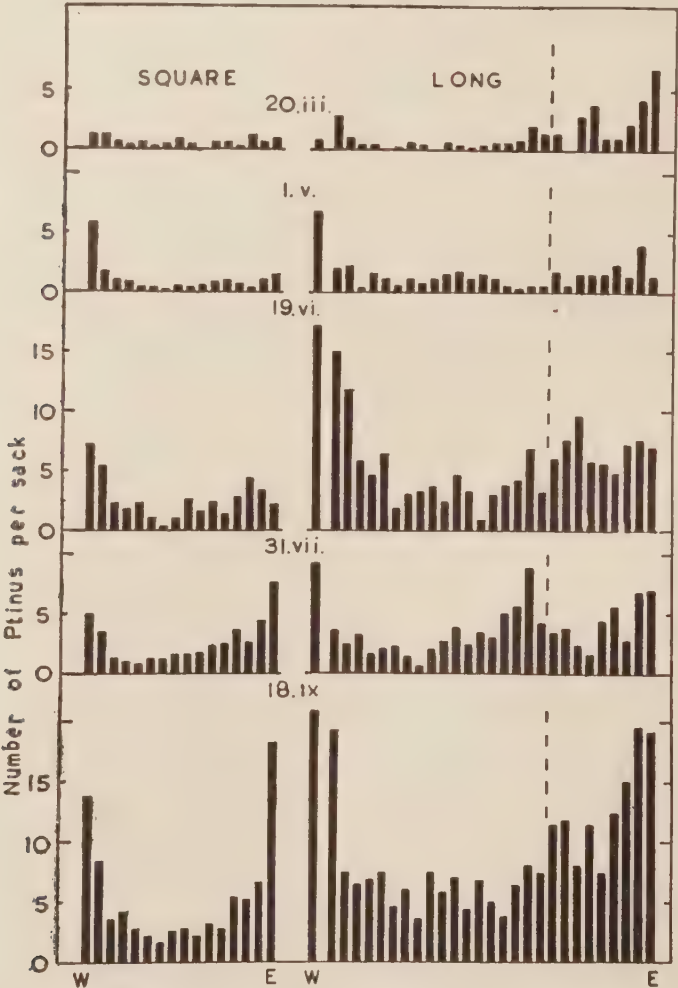


Fig. 6.—Mean number of adult *P. tectus* per natural row (16 sacks each) in the square section of Room 70 and across the natural rows (5 sacks) in the long section for each examination. At the west end of the long section is a natural row of 7 sacks (see fig. 2). The dotted line indicates the inside position of the tarpaulin which covered the eastern 9 sacks of the long rows.

The left-hand side of fig. 6 illustrates the changes in the total numbers found in each natural (*i.e.* N. to S.) row of the square section. The consistent predominance of insects on the outer rows is well shown but is least marked on the earliest visit.

The right-hand side of fig. 6 illustrates the changes in total numbers across the natural rows (N. to S.) of the long section and shows a similar predominance of beetles at the ends of these rows. An extra region of high density is revealed at the tarpaulin edge. On 19th June the population of the outer row of the long section was lower than that of some of the inner rows.

Distribution of larvae within sacks of flour.

Laboratory experiments were made to determine whether feeding larvae could penetrate to the central core of a bag. Small bags holding about 1 kg. of flour, measuring about 4 ins. × 10 ins. × 5 ins., when full were used. By means of a glass cylinder 8 ins. long a central core of flour stained red (with alcoholic safranin) was placed in each bag. The red core was surrounded by a layer of ordinary flour 1 in. thick. Feeding larvae (late first-early second instar) were introduced at the top and the bag closed. The bags were left at 23 C., 70 per cent. R.H., for 14 days when the larvae were removed by sieving and examined immediately. The gut either in the thorax or at the tip of the abdomen of some 60 per cent. of the larvae showed a red colour indicating that feeding on red flour had taken place. This shows that larvae can penetrate at least an inch from the flour surface. Feeding larvae can, of course, be seen readily in glass vessels at the bottom of cultures some inches deep, but these larvae are at the edge of the container. It is worth noting that about 30 per cent. of the flour in the 140 lb. sacks is within an inch of the sacking.

Effect of sacking material on distribution.

The flour sacks in both rooms in 1945 were made of two types of material, the majority of a fairly light and close mesh jute, the others of a close weave cotton. During most examinations a strong impression was gained that, on the average, more *Ptinus* adults were found on cotton sacks than on jute sacks. It is unlikely that any were missed although the insects were more easily seen on the cotton sacks.

The data for Room 69 show that there were more insects on the cotton sacks, but there were more cotton sacks in proportion towards the edges of the stack (fig. 1). An analysis of these data, allowing for the higher numbers of beetles at the edge of the stack, gave results suggesting a real preference for cotton sacks. This was not, however, definitely proved. The effect is certainly not great and an attempt to establish the point gave a similar indication of a slight preference for cotton.

Dispersal of marked beetles.

The method by which beetles were marked with paint is described on p. 392. When marked adults were set free among the sacks, wandering was restricted and recovery high, but when marked adults were freed on an open floor there was immediately a great deal of wandering and only a small proportion were recovered. Some adults evidently fell through holes in the floor, or crawled down posts, for several marked specimens were found on lower floors.

In the middle of Room 69, 100 marked adults were released on 29.v.45; 52 were retrieved one week later, the maximum distance covered being 7 sacks; in three weeks one adult reached the nearer side of Room 70. Of the 52 marked adults recovered, 33 moved along the "natural rows" towards the lighter side of the room, presumably because the looser packing between adjacent natural rows made movement easier. There seemed little difference in direction of movement across the rows, 28 going E. and 24 W. Marked beetles were liberated in the same spot on 26.ix and on this occasion specimens were recovered six days later at the edges of the bulk. The blue markings did not show up well and only a quarter of the releases were recovered and recognised, but these had moved further from the point of liberation.

Several releases, totalling about 500 in all, were made in 1946 on the open floor of Room 69 and recoveries were made from all parts of the room. In room 70, the predominant movement was away from the W. wall except for releases made a foot or so from it. The marked adults seen were not collected but recoveries were very small, usually less than 3 per cent. on each visit. Only about 1 in 300 of the beetles seen during 1946 had been marked.

Factors affecting activity.

This investigation throws some light on the activity and movement of adult *P. tectus* in the warehouse. Activity has an obvious bearing on the ease with which the insects can be found, on their migration and oviposition, on trapping and on the effect of insecticides. Important laboratory investigations on the behaviour of this species have been made by Dr. Gunn and his colleagues. Most of their work was on the activity of the adults measured as the proportion of insects moving at a given instant. The factors affecting activity which they investigated were light, temperature and humidity. Bentley & others (1941) showed that in alternating light and darkness, maximum activity occurred during darkness. In normal conditions of variable temperature, but in constant light, greatest activity occurred when the temperature was falling or was low (10–15°C.). Activity was reduced when food was present.

Gunn and Hopf (1942) showed that the amount of activity was fairly steady at constant temperatures between 15 and 35°C. but at any given temperature activity was greatest in those insects which had previously been under warmer conditions. With the temperature changing at least 3°C. an hour there was a great increase in activity especially when the temperature was falling. With a rising temperature, maximum activity occurred at 10–15°C. A change of 3°C. per hour is rather more than normally occurs in Britain, but it may happen in small local areas.

Bentley (1944) found that desiccated animals were most active at high humidities, but as the insects became thirsty the activity at low humidity increased.

These observations, on the whole, fit closely with the warehouse observations except possibly the last, since little activity was noticed at low humidities during daylight. Recently a bin containing bags of wheatfeed infested by *P. tectus* has provided interesting data. The windows of the building were closed at night, resulting in low humidity, but were opened at 8.00 G.M.T. This reinforced the slight rise in humidity which normally accompanies the rising temperature at this time of day and on one occasion a rise from 50 to 80 per cent. R.H. occurred. By 10.00 G.M.T. the activity of the *Ptinus* in the bin was extremely marked but had ceased by 12.00 G.M.T. when the humidity had fallen to 40 per cent. This observation was repeated whenever a rise of humidity could be induced, and it was found that the effect was most marked at high humidity levels. At these times the temperature was at 20°C. rising about 1°C. an hour, and the light was very bright, but a reaction was observable in a quarter of an hour.

In the warehouse these reactions to environmental conditions cause marked activity at night, when darkness, falling temperature and increasing relative humidity all tend to increase the proportion of active insects. Thus adults were active every night (see also Ewer, 1943) unless some unfavourable factor prevented activity. The main factor inhibiting activity was low temperature. Observation has shown that mass activity of adults occurs at 3°C. but not at 2°C. or lower although even then a few moving individuals can be seen. Clean produce placed in infested premises can be invaded even when overnight temperatures are about 2°C. The beetles will remain at the base of the sacks until they are active enough to climb, so that in cold weather the proportion at the base may be large. The presence of food reduces activity and sacks of food are obstructions which reduce the ease of movement over the floor. In consequence the movement in empty rooms exceeds that on loaded floors.

There is no clear evidence on the effect of low humidity. Bentley (1944) found that prolonged exposure to very low relative humidity reduced activity for a week and was followed by a much increased activity. Low daytime humidities (in the region of 40 per cent.) occur fairly frequently in British warehouses but a sustained low humidity, including night periods, is infrequent and seldom lasts for longer than a week. Such periods probably cause reduced activity and prevent egg laying. The adult females require drinking water if eggs are to be laid freely at low humidity; in this warehouse, water came only from rain and was thus not available in dry periods. Bentley records that *Ptinus* adults kept without water for ten days gained 17-29 per cent. in weight when allowed to drink. Thirty-eight adults collected in this warehouse on 5.vi.45 (not an exceptionally dry period) gained 18 per cent. in weight when allowed to drink, so they had probably been without free water for some time.

Conclusions : Chief aspects of distribution in a warehouse.

Insects may invade stored produce from the endemic warehouse population or, being present on the produce on arrival, increase in numbers during storage. In the single-tier stacks examined, the distribution of adults was largely peripheral and it seems likely that this is the normal adult distribution whatever the source of insects. Central sacks are only likely to be heavily infested when the whole stack is riddled or if a heavily infested bag is put there when stacking. Such a sack would be a focus of infestation which would spread outwards quickly. Examination of a few multi-tier stacks suggests that peripheral distribution, both vertically and horizontally, also prevails in these. Extension of these tendencies suggests that in the outside faces of a stack (four sides, top and bottom) the highest densities would occur at the corners and edges; the core of the stack would be the least infested.

The face which rests on the floor is the most accessible to the endemic population of the floor cracks and will be darker than the exposed faces. There will be suitable crannies for insects on both sides of the sacks of this layer but movement may be difficult. The presence of even a single tier of food sacks makes movement slow. Movement depends on the conditions prevailing during darkness. In 1946, for instance, it was not until March that substantial numbers of the insects from the warehouse moved on to bags of clean material. In both years a fairly steady proportion (30-40 per cent.) of the population of beetles associated with the sacks or bags was found near the floor from March to October, regardless of the total population. Thus it is possible that even in a large stack the proportion near floor level would become static when the whole stack had reached a definite (probably low) density of infestation.

It is clear that the general picture of distribution may be modified by local conditions such as draughts, wet patches and dark spots. Bentley (1944) has shown that the reactions of *Ptinus* to humidity vary according to its need for water; it reacts positively if thirsty, and negatively otherwise. Generally in this warehouse the adults were thirsty so that leaks and wet patches were attractive. If they reacted negatively to water after drinking the fact was not obvious, for the wet areas were always the most densely populated.

In Room 70, abrupt changes of weather caused changes in the population distribution. The W. side of the bulk was subjected to drying out by wind and bright sunshine and wetting by rain through broken windows. Visits on 1.v and 19.vi.45 showed a major part of the infestation at this edge, but a visit on 31.vii showed a much lower proportion and an actual decrease in numbers. The numerical increase between 20.iii and 1.v could be accounted for by emergence of insects from hiding and from the flour; by 19.vi some of the new generation could be emerging—there was some increase everywhere in the room. On 31.vii, which was cold, a fall in the

numbers found shows that there was some return to hiding places, and an increase on the lee side of the room suggested a migrating drift. At the S.E. edge, an area of nearly 50 sacks of flour, covered by a tarpaulin to protect them from rain, was always one of the most densely populated parts, as it was always dark and possibly the humidity was high. Even here the population fell on 31.vii, and again migration was the likely cause. It is interesting to note that there was a persistent "edge effect" at the limit of the tarpaulin.

In Room 69, where conditions were more stable, there were no such violent population changes. The N.W. corner, which let in the rain, showed the highest density of population.

Another factor which affects local distribution is the presence of suitable crevices. Upholstery tacks in the floor, wide floor cracks, and a generally uneven surface led to an irregularity in the distribution of the sites suited to the adult. As a result the proportion of adults under the two sets of small bags in 1946 differ by a consistent if small percentage. The presence of rubbish, sacks, machinery, etc., increases the number of these sites and so of course does the stored food.

Adults lay their eggs through the sacking into the surface layers of food in the sacks or in the food on the floor. The larvae do not seem to move far from the place where they hatch and no larvae were obtained in samples from the middle of sacks. Fully fed larvae inside sacks generally spin cocoons and pupate on the sacking and the adults generally emerge to the outside of the sack. Otherwise the adults do not readily pass through sacking materials except those of very loose mesh; larvae penetrate more easily than adults. Both adults and larvae emerge from the sacks more easily than they enter. Larvae feeding on the floor wander in search of a pupation site even in exposed light positions.

In the empty warehouse, larvae spun in cocoons were found especially in areas where the previous population was densest, for example, in the position formerly occupied by the stack. The mobile adults soon dispersed to darker and more secure places, particularly the cracks at the wall-bases, under machinery and in the subfloor space.

Notes on Methods for Rapid Estimation of Numbers present in a Warehouse.

When it is necessary to show the presence of *Plinus* it is safe to examine only the outer sacks of a stack, especially those near corners and those in dark or damp parts of the warehouse. Owing to the greater activity of the adults in darkness it is usually easier to find them by inspection at night.

An estimate of the number of beetles associated with the stored materials could be made by counting the beetles on the outsides of all the sacks, but the labour involved would generally prohibit the use of such a method. Even random sampling would involve so much labour in moving large numbers of sacks in order to reach the chosen sacks that it would be impracticable.

The nature of the peripheral distribution, however, renders it possible to make a more or less reliable estimate of numbers by examination of external sacks only. In both of the rooms studied the mean number of insects found on the outermost sacks of the stack was about double the mean for all sacks. If this proportion is assumed to be general, the estimate of the population on the sacks in a single tier can be obtained as follows. First the sacks in the outside rows of the stack are counted. Then the number of sacks to be examined must be decided; a convenient proportion would normally be about 5 per cent. of the total sacks in the stack. The sacks to be examined should be selected from the four sides approximately in proportion to the numbers in the sides; to reduce the labour of shifting the sacks, they should be taken in groups of 4 or 5 adjacent ones.

Since the beetles tend to congregate towards the ends of the outside rows, it is necessary to select samples equally from the various parts of the rows. Hence each row is divided into five nearly equal parts: two ends, a middle, and two intermediate sections. With a rectangular stack, there will thus be twenty sections in all. The total sample must be taken from at least five of these—two ends, one middle, two intermediate—and should be fairly evenly spaced around the periphery.

In a narrow stack there may be less than 20 sacks in a row; in such a case it is not convenient to divide the row into more than three sections. In a very long row, each of the 5 sections into which it is divided may contain many more than the 4 or 5 sacks needed for sampling. In an end section of such a long row, it is important to take the sample from the true end of the row.

These principles were applied to the results obtained from Room 69. Since the population present on all the sacks on six different dates is known, it is possible to check the accuracy of the above method of estimating the total population on the sacks by choosing sample sacks in the way described. The samples have in fact been chosen only from the three straight borders of the stack, the irregular southern edge being avoided.

The sample size adopted is 20 sacks, comprising 5 evenly spaced groups of 4 adjacent sacks. One such sample taken from the data of 14.iii gives an estimated mean population per sack of 1.00 beetles, whereas the mean calculated from the total count was 0.68. The error is thus +47 per cent. Five parallel estimations have been made by selecting 5 analagous samples from the data. The percentage errors of these estimates are +76, +69, +40, +7 and -3 per cent. This procedure has been repeated with the data for the other five dates. The errors of the 36 estimates obtained are shown in Table III. They range from -26 per cent. to +99 per cent. of the figure based on the total count; 12 of the 36 estimates are within 10 per cent. of the total count figure, 21 within 20 per cent. of it, and 33 within 50 per cent. of it, 3 being more than 50 per cent. out.

TABLE III.

Percentage deviation of estimates, based on samples of 20 sacks, from the actual numbers of *P. tectus* adults as determined by counting the beetles on all the sacks.

Sample	% deviations of estimates based on data of—						Mean B
	14.iii	14.v	5.vi	3.vii	27.viii	2.x	
1	+47	+11	-19	+1	+8	-12	+6.0
2	+76	+9	+99	+14	+26	+23	+41.2
3	+69	-13	+26	+20	-3	-10	+14.8
4	+40	-6	-26	-9	+13	-17	-0.8
5	+3	+24	+44	+36	+33	+22	+27.0
6	+7	+13	-25	-8	+5	+7	-0.2
Mean A ...	+40.3	+6.3	+16.5	+9.0	+13.7	+2.2	

Each sample is taken in relation to the data for six examination dates, and the mean percentage deviation of the six estimates based upon each sample is shown in column B. Line A gives the mean percentage deviation of the estimates based on six analagous samples of the population on each examination day.

Four of the estimates obtained from the data of 14.iii are particularly high, probably because of the relatively low population at that time (*cf.* fig. 3). All the estimates from samples 2 and 5 (Table III) are also high, because these samples include sacks from the heavily infested N.W. corner. Such highly localised concentrations of animals are best avoided in sampling when possible, since they can give rise to gross over-estimates.

An attempt has been made to establish a relationship between the variance of the mean for the outer row of sacks and that of the mean for the whole stack, but no consistent relationship has been found. In the absence of an estimate of variance it is not possible to arrive at a reliable figure for the error of an estimated mean, but it is probably within 50 per cent. of the true mean. Just what degree of accuracy can generally be expected from the procedure described above, only further experience can show.

The above estimations of numbers on the sacks takes no account of those insects which were hidden in the fabric of the building. The difficulty of finding adult *Ptinus* in empty premises indicates that the proportion hidden may be large.

In empty premises, cracks in floors and walls may be examined. The probing of cracks could be made the basis of a numerical estimate by examining a known proportion of the total of such sites but the process is very laborious and often unrewarding, because the beetles are inaccessible in such places as the narrow cavities at the junction of floor and walls.

These considerations emphasize the impossibility of collecting and counting the whole population, or even of getting a representative sample of it by ordinary collecting methods. It is necessary to turn to indirect methods, such as trapping, or the release of marked specimens.

Attempts were made to trap beetles in February, 1946. Since it was noticed that beetles tended to congregate under the edges of a tarpaulin on the floor of Room 70, sheets of newspaper were put down, and sheets of organdie about a yard square were also pinned to the floor. Neither of these media proved successful and it was concluded that a heavier and more opaque material was needed. It is clear also that the low temperature at the time contributed to the failure. Later, when sheets of newspaper carrying piles of food materials were put down to provide an oviposition site, numbers of beetles collected under the paper. This may have been due to the attractive odour of the foods, or to the extra weight and opacity caused by their presence on the paper.

Trapping alone cannot provide an estimate of the total population, although it provides a means of comparing the populations of similar rooms. It is ineffective for adults below 2°C., at which temperatures *Ptinus* becomes inactive. Trapping is very much more effective in empty than in loaded premises.

An estimate of the number of insects in a room or warehouse may be made by marking a known number of insects and noting the proportions marked and unmarked among those which are found on subsequent occasions. The interpretation of results from this procedure, however, is not easy, particularly when the recovery of marked animals is very small. In the present investigations (Room 69) only about 1 in 300 of the beetles found in the empty room were marked specimens. The number of marked specimens found beneath the traps was generally about 2 per cent. of the number released. This gives two estimates of the population each of about 150,000. In Room 69 when loaded, the recovery of marked specimens was of the order of 50 per cent. one week after release. This suggests that 50 per cent. of all adults were found, but since wandering is known to be slow in a loaded room it is likely that many of the marked specimens failed in the short period of one week to reach all possible hiding places. Hence the proportion of the total population found in the loaded room is probably less than 50 per cent.

Cellulose paints were used for marking the beetles. They proved permanent though difficult to apply. The paint was applied with a mounted needle, the point of which was dipped into the undiluted paint and placed against the elytra. The insect could then be lifted over to a piece of blotting paper, which it would grasp and so walk away with a spot of paint on its back. It was not easy to mark the

same insect more than once. White and yellow were the most useful colours since they could readily be noticed in the darker parts of the warehouse. Bright green, orange and red were also suitable, but dark colours could only be distinguished with difficulty from "rubbed" specimens which were rather common. Adults marked were normally laboratory specimens about one week old.

Marked specimens did give useful information on wandering and longevity, but were of no great value in estimating population.

Summary.

An account is given of observations made in two rooms of a warehouse during 1945 and 1946. In 1945 fortified flour was stored in a single tier of upright sacks, whilst in 1946 the rooms were empty except for 50 small experimental bags of wheatfeed.

Before the flour arrived in 1945, no insects were found except a few larval *Ptinus* in a bag of sweepings. Adult *Ptinus tectus* soon appeared on the sacks of flour and, in one room, the numbers on the sacks increased steadily to 6,000 by the time the flour was removed in November. In the other room, where the ecological conditions were obviously variable, the increase was erratic, but there were 4,500 adult *Ptinus* on the sacks by the time the flour was removed. The numbers on the experimental bags in 1946 rose from zero to 5,000 by the end of September. The weekly figures varied with temperature, falls in numbers coinciding with colder weather. In both years a steady 30-40 per cent. of the total *Ptinus* on the sacks or bags were found at the bottom except for the early months of 1946 when 80 per cent. were at the base of the bags until April.

In empty premises it was obvious that many adult beetles hid in the fabric of the building, a rough estimate of 150,000 being obtained following the use of traps and marked beetles. There were indications that the main hiding place in this building was at the junction of walls and floor. After the sacks of flour had been removed in 1945, it was estimated that the flour covering the floor of Room 70 contained about 7,000 larvae. These would normally be removed by sweeping and vacuum cleaning, but this treatment left behind about a further 10,000 larvae in the floor cracks of each room. No estimate of larvae inside the sacks was possible, but in eight months in 1946, about 1,500 eggs were laid in each small bag of wheatfeed. An average of at least 300 eggs per day were laid in the room in 1946.

Ptinus tectus has two complete generations a year. All stages may survive the winter so that overlap of generations is complete.

In a stack of sacks of flour, adults are most abundant on the sacks at the edge of the stack especially on those near the corners. Draughts, wetness and sunshine caused local changes of distribution. There was some suggestion that *Ptinus* preferred to rest upon cotton sacks rather than on jute. Larvae are normally present only in the outer layers of flour in a sack and spin cocoons on the inside surface of the sacking. The adults emerge through the sacking to the outside.

The activity of *Ptinus* in a warehouse is greatest during darkness. Hence it is fairly continuous in dark premises and periodic in light places. In the latter, beetles emerge from their hiding places at dark and return at dawn. If dark crevices are provided artificially they act as traps, for some of the beetles will use them as daytime hiding places. Activity is reduced by low temperatures but does not stop altogether until it is as low as 2°C.

Two methods are suggested for actual estimation of insect numbers without too much labour. One for estimating the numbers on the sacks is based on the fact that most of the insects are found on outside sacks, and involves the counting of the insects on several representative groups of contiguous sacks. Over half of the

estimates made by applying this method to the data from one of the rooms in 1945 were within 20 per cent. of the correct values as indicated by total counts of the population. The combination of traps with the release of marked beetles is suggested for estimating the resident population of empty premises.

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References.

- BENTLEY, E. W. (1944). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. V. Humidity reactions. —J. exp. Biol., **20**, pp. 152–158.
- BENTLEY, E. W., GUNN, D. L. & EWER, D. W. (1941). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. I. The daily rhythm of locomotory activity, especially in relation to light and temperature.—J. exp. Biol., **18**, pp. 182–195.
- EWER, R. F. (1943). Diurnal activity of three insect pests of stored products.—Nature, **152**, pp. 133–134.
- GUNN, D. L. & HOPF, H. S. (1942). The biology and behaviour of *Ptinus tectus* Boie. (Coleoptera, Ptinidae), a pest of stored products. II. The amount of locomotory activity in relation to experimental and to previous temperatures.—J. exp. Biol., **18**, pp. 278–289.
- OXLEY, T. A. & HENDERSON, F. Y. (1944). The properties of grain in bulk. I. Instruments for making measurements in grain stored in bulk.—J. Soc. chem. Ind., **63**, pp. 48–51.
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A METHOD OF DISTINGUISHING THE LARVAL STAGES OF *AGRIOTES SPUTATOR* (L.) (COL., ELATERID.).

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Little was known about the composition of wireworm populations until Salt and Hollick (1944) published an account of the complete wireworm populations of soil samples from pasture. Their paper showed for the first time the great preponderance of small larvae in any wireworm population from old grassland. It also pointed out that there is "in general, a very marked correlation between the length of the larvae and their numbers—there are many larvae in the smaller size-groups, and progressively decreasing numbers of the larger sizes".

The question at once arises: In such a wireworm population what is the relation between the size of the larvae and their age? It is known that the larval life extends over a period of about four years, but apart from the fact that in general the smallest larvae are the youngest, and the largest are the oldest, it is impossible to say what proportion of the larvae in a population is in the first, second, third or fourth year of their life. If the population could be accurately divided into year-groups, or into instars, it would be possible to trace the incidence of favourable or unfavourable factors. For instance, a population containing an especially high proportion of larvae in the second year-group would point to especially favourable conditions for oviposition two years previously, and for survival since.

The primary object of the present investigation was to discover a means of distinguishing with certainty the different instars of wireworms as the first step in the analysis of larval populations with respect to age and time.

Material.

The investigations were confined to *Agriotes sputator* (L.) from Cambridgeshire and, for the greater part of the work, it was desirable to study a large homogeneous collection of larvae. Such a collection was obtained from a soil sample, of area 1 yard sq. and depth 6 ins., taken from permanent grassland in August 1942. This yielded 1,039 wireworms.

The larvae from a second sample were examined to corroborate the data obtained from this collection. This sample, from an area 1 foot sq. and 18 ins. deep, was from the same field but had been taken in February 1942. The larvae were extracted from the soil samples by the method described by Salt and Hollick (1944). They were preserved in 70 per cent. alcohol.

A number of cast skins from larvae reared individually in tubes in the laboratory was also available. Some of these larvae were known to have pupated.

The character used to distinguish *A. sputator* larvae from other species present in the August collection was that given by Guénat (1934), that is the punctation on the cushion on which the legs are borne. This character was easily seen on the large larvae using a 12.5 ocular and a 6.8 objective, but not on the small specimens; all but three of a total of 755 large larvae examined proved to be *sputator*. The rest of the collection were all less than 4.8 mm. in length. It would have necessitated mounting each specimen in glycerine and using a much higher magnification to have determined the species in each case, and this would have involved a very considerable amount of time. As the percentage of large larvae which were not *sputator* was so small, it

was decided that it was unnecessary to examine further the small ones and that it could be assumed that the number of larvae in this group which were not *sputator* would be negligible. Only one of the 113 larvae in the February collection was found to be other than *sputator*; 30 small larvae were not examined.

Analysis of Material.

Total length measurements.

The material used for the first and major part of the work was the August collection of 1,039 wireworms. In order to obtain some idea of the composition of this collection, the larvae, extended to their greatest length, were measured by means of an ocular micrometer scale each division of which was 0.4 mm. The larvae were thus divided into size-groups, the range of length of the larvae within each group being 0.4 mm. The results are given in fig. 1.

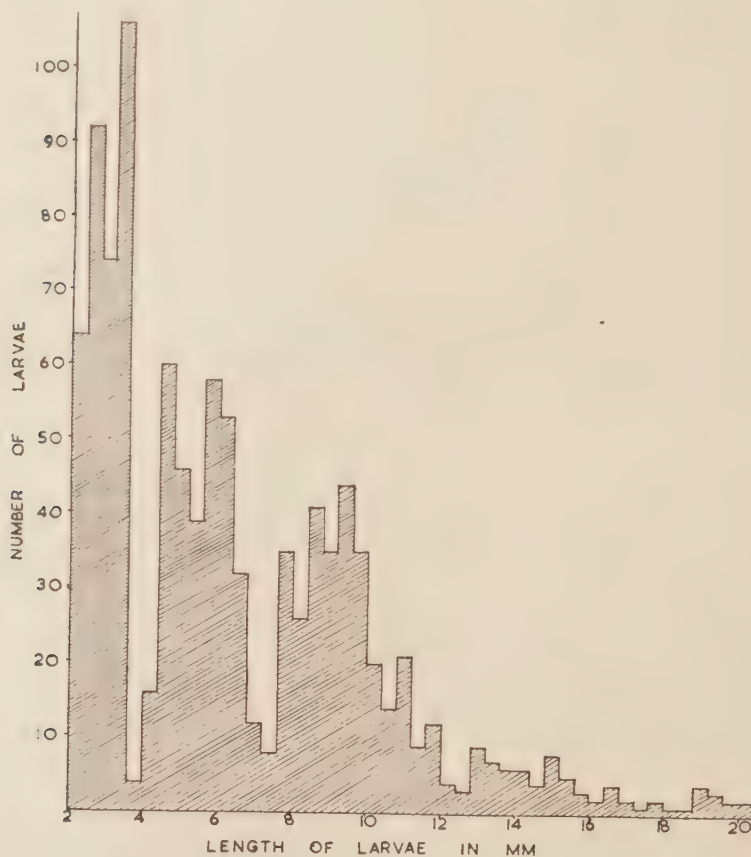


Fig. 1.—Summary of total length measurements of 1,039 larvae of *Agriotes sputator* from the August square yard sample.

This histogram shows two things. First that the population consists, as was expected, of a preponderance of small larvae and decreasing numbers of larger ones; and secondly that the larvae can be divided into four principal size-groups. The groups are sufficiently well defined to suggest that they represent the four years of

larval development. The limits of the groups are obscure, but larvae between 2.0 mm. and 3.6 mm. might be supposed to be in their first year; those between 3.61 mm. and 7.2 mm. in their second year; those between 7.21 mm. and 12.4 mm. in their third year; and those between 12.41 mm. and 20.4 mm. in their fourth year. Further, the tendency of each of these groups to be subdivided into two parts, particularly groups I and II, suggests two instars for each year.

It is obvious, however, that any division of the larvae into year-groups based only on total length measurements must necessarily be very tentative. The value of such measurements for the determination of instars is practically nil, and the need for some more conclusive character is manifest.

Other measurements.

As wireworms of obviously different ages showed no striking qualitative differences, an attempt was made to separate the instars of *A. sputator* by rigorous measurements of some of the chitinised parts of the larvae.

From the measurements of total length, shown in fig. 1, it seems reasonable to suppose that most of the larvae of a length less than 3.6 mm. would belong to an earlier instar than those of a length more than 4.01 mm. However, there may be in the later instar some abnormally small larvae, and some unusually large ones in the earlier instar. Also, it is impossible to tell to which instar the larvae measuring 3.61 mm.-4.0 mm. belong. A number of larvae from this area of the graph was selected for preliminary investigations.

The greatest width of the head capsule of each of these larvae was measured to the nearest 0.04 mm. by means of an ocular micrometer. The groups into which these head measurements fell, however, were still not well defined and, in this region of the graph, were little more precise than in the case of measurements of total length. Similar unsatisfactory results were obtained by using the length of the prothorax, and the width and length of the last abdominal segment. Measurements of the mandibles were also found to be unreliable as these mouth-parts become considerably worn away with use.

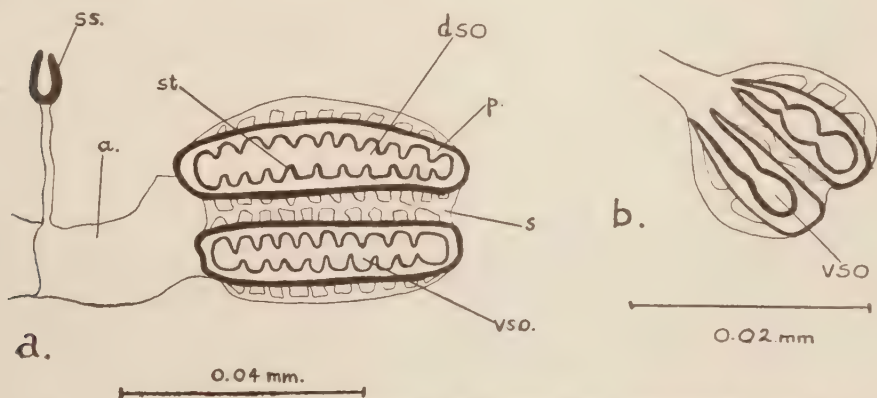


Fig. 2. (a) Spiracle of *Agriotes sputator* larva from fourth abdominal segment, left side. (b) Spiracle of first growth stage larva with a single tooth on each side of the ventral orifice represented by a thickening of the peritreme.

a	...	atrium
dso	...	dorsal stigmatic orifice
p	...	peritreme
s	...	septum
ss	...	stigmatic scar
st	...	spiracle tooth
vso	...	ventral stigmatic orifice

Quantitative differences in the teeth of the spiracles.

It was noticed by Rymer Roberts (1921) that in *Agriotes obscurus* (L.) "the corrugations which project as small teeth on either side of the stigmatic orifice vary in number according to the instar". In 1922 he gave examples of the number of teeth on the thoracic and abdominal spiracles of *A. sputator* larvae in what he thought to be the first, third and final instars. This character seemed to offer a more promising means of distinguishing the instars of these larvae.

The spiracles of *A. sputator* are 18 in number. Two are situated on the mesothorax and two on each of abdominal segments 1-8. The mesothoracic spiracles are always larger than the abdominal ones, but otherwise similar in structure. They lie on the pleura of the mesothorax almost parallel to the body axis. The abdominal spiracles lie near the lateral border of the tergite in the anterior one-third of the segment, and at an angle of about 30 degrees to the body axis.

All the spiracles are biforous (fig. 2), each orifice being surrounded by a well defined peritreme. The more dorsal orifice is usually the slightly larger of the two. The peritreme which surrounds the orifice is ridged, and these ridges project into the orifices to form four rows of minute teeth. The two rows of teeth on each orifice of any spiracle always have the same number, but the rows of the larger orifice have a slightly higher number than those of the smaller one.

A comparison of the spiracles on two successive cast skins from one *A. sputator* larva showed that the number of teeth around both orifices was increased after the moult, and it was thought that by counting these teeth on a large number of larvae it would be possible to establish a means of dividing the larvae into groups.



Fig. 3a.—Average numbers of teeth on the two thoracic spiracles of the larvae of *Agriotes sputator* from the August square yard sample.

The number of teeth on the spiracles of individual larvae, however, varied considerably. The number on the mesothoracic spiracle was always larger than on any abdominal spiracle, but not by any constant amount. The abdominal spiracles did not bear any constant relation to one another; the number of teeth on the eighth abdominal spiracle was always slightly higher than that on any one of the seven others, but the difference between this and the other spiracles was not exactly the same for different larvae.

It was decided that all the abdominal spiracles must be examined. Since the two rows for each orifice had the same number of teeth, but the number in the rows of one orifice was greater than that in the other, it was necessary to count the teeth on only one row from each orifice. Thus by counting the teeth on two rows from each of the 16 abdominal spiracles and taking an average of all these numbers, the average number of teeth per row on the abdominal spiracles of each larva was obtained. As the number of teeth on the thoracic spiracle was always considerably more than on any abdominal spiracle, the teeth on one row of each orifice of these were counted, and a separate average for the number of teeth per row on the thoracic spiracles was found. Any spiracle which was obviously abnormal in shape was ignored, and the average number of teeth per row on the other spiracles of the larva was calculated.

Such counts were made on larvae from the August collection, irrespective of size. Each larva was mounted in glycerine under a coverslip and examined under the high power of a compound microscope.

It was soon apparent that the number of spiracle teeth varied with the size of the larvae; the older and larger wireworms had more teeth than the smaller, younger ones. The number of teeth, also, was not constant for individuals of any particular size and further wireworms in different size-groups might have the same number of teeth.

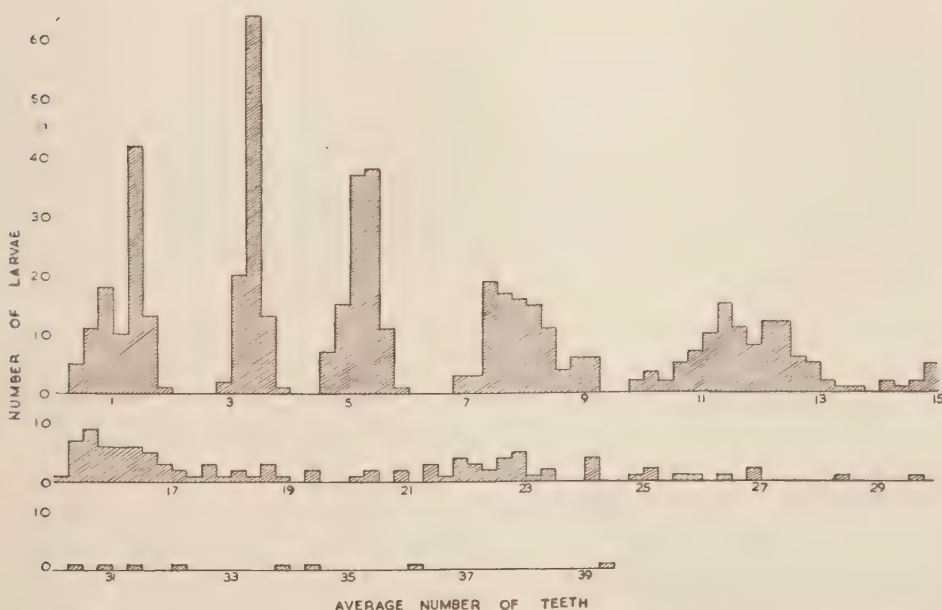


Fig. 3b.—Average numbers of teeth on the 16 abdominal spiracles of the larvae of *Agriotes sputator* from the August square yard sample.

As the work proceeded, the tooth numbers were seen to be falling into eight groups. Moreover, these groups were remaining discrete, both those formed by the number of teeth on the thoracic spiracles, and those formed by the numbers on the abdominal spiracles. It was hoped to count the teeth on the spiracles until each group contained at least 100 larvae, but, from the August collection, this was possible for only the first five groups. The frequency with which each average number of teeth on the thoracic and abdominal spiracles occur is shown in the histograms of fig. 3 (*a* & *b*). It can be seen that the first five groups into which the number of teeth fall are in all cases discrete. As would be expected, when the spiracle is larger, with a corresponding increase in the number of teeth, the range of numbers within the group also increases. The numbers of larvae in the other groups, particularly in groups VII and VIII, where there were only 43 and 9 larvae respectively, are not large enough for any conclusive results to be obtained from them. These three groups will be reconsidered later.

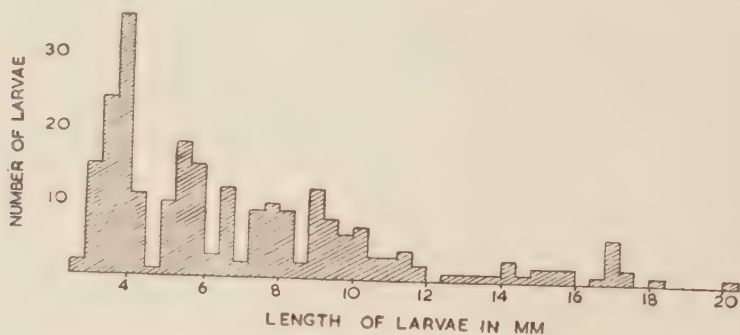


Fig. 4.—Summary of total length measurements of larvae from the February square foot sample.

Larvae from other collections.

So far the work had been carried out exclusively on larvae from the August sample. In order to see if the same conditions held true for other times of the year, a sample was chosen which had been taken in the winter. This sample, comprising 243 larvae taken in February of the same year, was first measured, and the resulting histogram (fig. 4) indicated that the composition of the population was similar to that of the August sample. The numbers of spiracle teeth were now counted in the same way as they had been for those of the August collection (figs. 5 *a* & *b*).

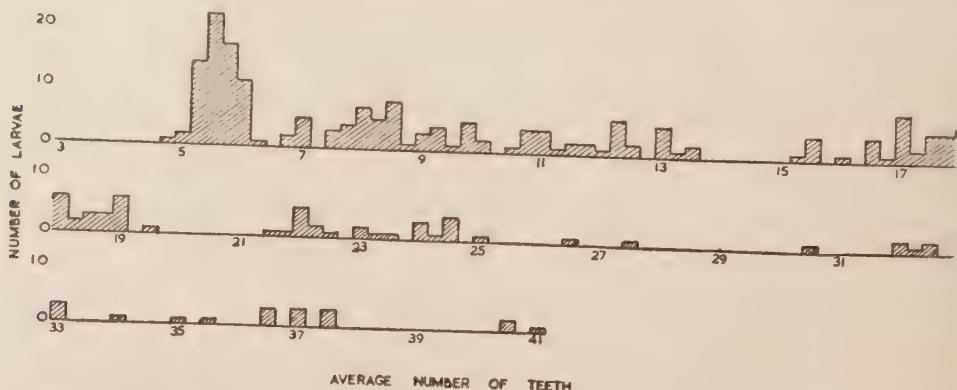


Fig. 5a.—Average numbers of teeth on the two thoracic spiracles of the larvae from the February square foot sample.

It will be seen from these figures that the numbers again fall in discrete groups, but for this collection there were only six, the first and last groups not being represented. A comparison of the range within each group formed by the numbers of teeth on either the thoracic or abdominal spiracles for the larvae of the August and February samples shows that the groups correspond very closely (Table I).

TABLE I.

Comparison of the range within each group formed by the numbers of teeth on the thoracic and abdominal spiracles for larvae of the August and February samples.

Group	Thoracic spiracles		Abdominal spiracles	
	Range on larvae from		Range on larvae from	
	August sample	February sample	August sample	February sample
I	3.0 - 4.0		0.25- 2.0	
II	4.5 - 6.0	4.75- 6.25	2.75- 4.0	3.0 - 4.0
III	6.75- 9.75	6.75-10.0	4.5 - 6.0	4.5 - 6.0
IV	10.5 -14.25	10.5 -13.5	6.75- 9.25	6.75- 9.5
V	15.25-20.75	15.25-19.5	9.75-13.75	10.5 -13.5
VI	21.5 -30.5	21.5 -30.5	14.0 -19.5	14.0 -19.0
VII	32.25-40.5	32.0 -41.0	20.0 -28.5	21.25-28.5
VIII	44.0 -62.0		29.5 -39.5	

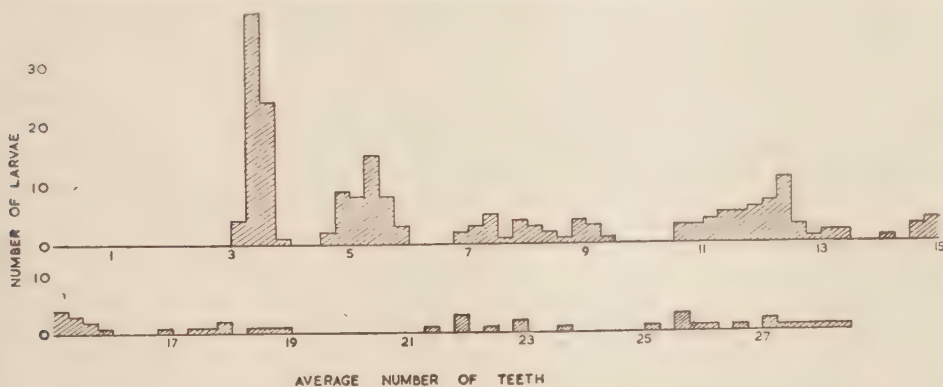


Fig. 5b.—Average numbers of teeth on the 16 abdominal spiracles of the larvae from the February square foot sample.

The limits of the groups formed by the numbers of teeth on the spiracles of the larvae of the February sample are very nearly the same as those of the groups of the August sample, and in most cases are within these latter. These closely corresponding groups, then, appear in summer, when the larvae are actively feeding and ecdysing, and in winter, when the larvae are neither ecdysing nor so active.

Large larvae from soil samples taken in any month of the year were now examined for additional data on the higher spiracle tooth numbers. The frequencies with which the different tooth numbers occur in these are shown in fig. 6 (*a* & *b*). The groups were not so distinct as they had been for the first five groups (fig. 3), as the numbers were spread over a much wider range, and it was thus more difficult to determine the exact limits of the groups. Where doubt occurred, as between groups VII and VIII of the thoracic spiracle tooth numbers, the thoracic count was plotted against the abdominal one for each larva in this area of the graph. In this

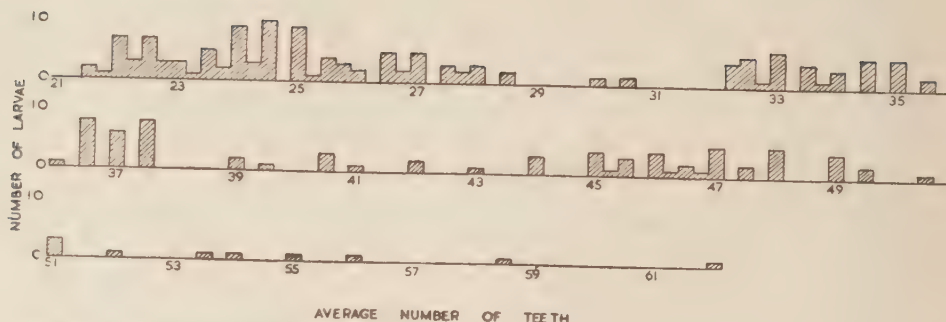


Fig. 6a.—Average numbers of teeth on the two thoracic spiracles of larvae with higher spiracle tooth numbers.

way it was clearly seen where the break between the groups occurred and the limits were fixed as follows :—

Group	Thorax	Abdomen
VI	21.5 -30.5	14.0 -19.5
VII	32.25-41.0	20.0 -28.5
VIII	42.0 -62.0	29.25-39.5

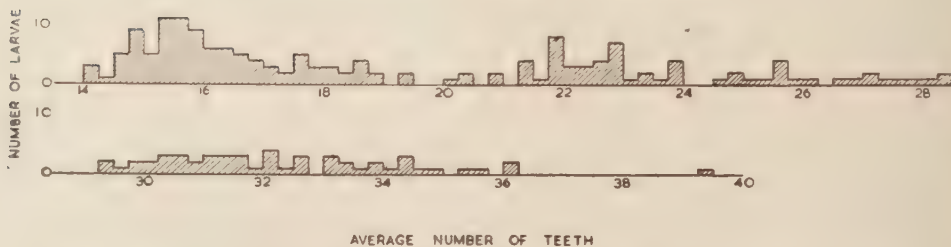


Fig. 6b.—Average numbers of teeth on the 16 abdominal spiracles of larvae with higher spiracle tooth numbers.

Thus by counting the teeth on the thoracic spiracles or on the abdominal spiracles of larvae of *A. sputator*, the wireworms can be divided into eight groups.

It has already been noted that there are more teeth on the thoracic than on the abdominal spiracles, and when the eight groups are considered together, an interesting relation becomes apparent. The number of teeth on the thoracic spiracles is nearly the same as the number on the abdominal spiracles of the next larger group. For example, the limits of group I in the thorax (3-4) approximate to those of group II in the abdomen (2.75-4). A similar relation for all groups can be seen from Table I and from the figures for groups VI, VII and VIII given above.

The variation in the number of spiracle teeth found on the larvae of any one group increases from group I to group VIII. This increase is accompanied by an increase in the variation of total length measurements of the larvae belonging to each of these groups (Table II). Thus in group I of the August sample the larvae were all between 2.0 and 3.6 mm., whereas they vary from 4.81 to 9.6 mm. in group IV and from 13.21 to 20.0 mm. in group VII.

TABLE II.

Range in length measurements for the larvae of each group.

Group	Length of larvae from August sample	Length of larvae from February sample	Range of length in larvae from August sample	Range of length in larvae from February sample
I	2.01-3.6 mm.		1.6 mm.	
II	2.41-5.2 mm.	2.41-4.4 mm.	2.8 mm.	2.0 mm.
III	3.21-6.8 mm.	3.21-6.4 mm.	3.6 mm.	3.2 mm.
IV	4.81-9.6 mm.	4.81-9.2 mm.	4.8 mm.	4.4 mm.
V	6.01-13.2 mm.	6.41-11.6 mm.	7.2 mm.	5.2 mm.
VI	8.41-16.4 mm.	8.41-14.0 mm.	8.0 mm.	5.6 mm.
VII	13.21-20.0 mm.	11.21-20.4 mm.	6.8 mm.	9.2 mm.
VIII	15.61-20.4 mm.		4.8 mm.	

The similarity between the lengths of the larvae in any one group for the two months is striking. Table II confirms the observations made at the beginning of the work on spiracles, that wireworms differing considerably in size could have the same spiracle tooth number, and that wireworms of the same size do belong to two or more tooth number groups.

Significance of the eight groups.

The question arises whether the eight groups into which the larvae fall when the teeth on their spiracles are counted represent eight larval instars. The following evidence indicated that this was so.

During the examination of the wireworms from the August sample, 55 larvae were found which were in the process of ecdysis, and on which the newly formed spiracle could be clearly seen lying beneath the old cuticle. The teeth on the old and the new spiracles of each of these specimens were counted, and in each case the average number on the new spiracle was within the limits of the group next above that of the old spiracle, this being true both for the thorax and the abdomen. Such larvae were found in groups I to VI ecdysing into groups II to VII respectively.

This was further supported by the examination of a number of cast skins collected from larvae each reared singly in a tube. When successive exuviae of 15 of these larvae were examined, it was found that the tooth number on the spiracles had passed from one group to the one next above. Some larvae had ecdysed only once, others twice before pupating or dying. The larvae were in groups IV to VII when first observed. This was not always so; other larvae reared in the same way as those mentioned above, were sometimes found to have cast their skins but to have remained in the same tooth number group. One larva had ecdysed four times; the first three moults gave the expected increase in tooth number but the last one failed to do so. Another larva, which also had moulted four times, gave the same tooth counts for each of the four exuviae, but the spiracles of the larva showed an increase in number after the fourth moult. This phenomenon occurred in seven larvae and at thirteen ecdyses.

In view of this, it could no longer be said that the *instar* of any particular larva could be determined by counting the spiracle teeth. Such a count would give no indication of the number of times the larva had moulted without increase in the number of teeth, or even whether it had behaved in this manner or not.

It was still possible, however, that the spiracle tooth numbers indicated the *growth stages* through which the larva passed in its normal development, and that in those cases in which there was no increase in tooth number, there would also be no increase in size. This was in fact found to be the case.

Two measurements were made to decide whether growth had taken place ; these were the length of the unstriated part of the dorsum of the prothorax and the length of the last abdominal segment from the posterior rims of the pits to the tip of the segment.

On measuring these cast skins, it was found that in those larvae in which there was an increase in the number of teeth, there was a corresponding increase in the length of these parts, such an increase indicating that growth had taken place at that particular ecdysis. In the case of those larvae in which the tooth number had remained the same before and after a moult, the size of these parts had also remained the same. In the case of the larva in which the number of teeth had increased at three ecdyses but not at the fourth, the two parts measured were longer after each of the first three ecdyses but had remained the same length after the last. The tooth number of one larva had stayed constant for three moults and then increased at the fourth, and the prothorax and last abdominal segment had correspondingly remained the same size until the fourth moult when they had increased in length.

In other words, whenever growth had occurred in the development of the larva, the number of teeth around the spiracle had increased from one group to the next. Thus the spiracle tooth numbers, although not indicating the instar, do show to which of eight growth stages the larva belongs.

It should be emphasised that this abnormal phenomenon of ecdysis without increase in size or tooth number has not yet been found to occur under natural conditions. None of the 55 natural ecdyses showed it, and only 13 of the 33 ecdyses of the reared specimens. It is most probable that this is an entirely unnatural condition and that normally instar and growth stage are in fact the same thing.

The soil sample taken in August was found to contain larvae in each of the eight growth stages, but that taken in February contained larvae in only six, there being none in either the first or the eighth growth stages. In the August sample only 0.85 per cent. of the larvae were in the eighth growth stage. Since pupation of *Agriotes sputator* in Cambridgeshire occurs from late June until the end of August (Salt & Hollick, 1944) the small percentage of larvae in growth stage VIII in the August collection and the complete absence of larvae in this growth stage in the February collection, suggest that it is after the eighth growth stage that the larvae pupate. This was found to be only partly true.

A number of large larvae of *A. sputator*, collected from an area of permanent grassland during June 1943, were kept isolated in tubes in the laboratory until they pupated. From these, 44 last larval skins were collected. When the teeth on the spiracles of these were counted, it was found that 28 of the larvae had reached the eighth growth stage before pupating. The other 16 skins were in growth stage VII, showing that the number of growth stages through which the larvae pass before pupating is not always the same.

Application of Analysis.

It has been shown that 700 individual larvae of *A. sputator* could be placed with certainty in the growth stage to which they belonged by counting the number of teeth on the two thoracic or on all the abdominal spiracles. This is too laborious for large scale work, and it was desirable to find the least number of spiracles which must be examined before a larva could be placed in its correct growth stage. The following quick method was found to be satisfactory.

First the teeth on the two outer rows of either thoracic spiracle are counted. If the average number of teeth per row falls within the range of one of the eight groups given in column 1 of Table III, the growth stage is determined as indicated. If this is not so, then the teeth on the outer rows of the other thoracic spiracle are counted, and the average for all four rows now counted tested against the groups in column 2.

If the growth stage still remains uncertain, recourse must be had to the abdominal spiracles 1-7, the teeth on the outer rows of one, two, or three of which can be counted and tested similarly against columns 3, 4, and 5 of the Table. If desirable, the teeth on the abdominal spiracles can be counted first and columns 3, 4, and 5 consulted. Should this not show to which growth stage the larva belongs, one or both of the thoracic spiracles should then be examined. All spiracles obviously abnormal in shape should be ignored.

TABLE III.

Table for determining the larval growth stages of *Agriotes sputator*.

Thoracic spiracles		Abdominal spiracles		
1	2	3	4	5
I 3-3.5	I 3-4	I 0-2	I 0-2	I 0-2
II 5-5.5	II 4.5-6	II 3-3.5	II 2.5-3.75	II 2.5-4
III 7-9.5	III 6.75-9.75	III 4.5-5.5	III 4.25-5.75	III 4.3-6
IV 11-13.5	IV 10.5-14	IV 7-9	IV 6.5-9	IV 6.5-9.3
V 15-19.5	V 15-20.75	V 10.5-13	V 10.25-13	V 9.8-13.3
VI 22-30	VI 21.5-29.75	VI 14.5-18.5	VI 14.25-18.5	VI 14-18.8
VII 32-39.5	VII 31.75-40.25	VII 20.5-27.5	VII 20.25-27.75	VII 19.5-27.7
VIII 42-62	VIII 41.75-62	VIII 30-40	VIII 28.75-40	VIII 28.7-40

It should be noticed that it is not advisable to count the teeth on abdominal spiracle 8, as this usually has a somewhat larger number of teeth, and an examination of it may result in the larva being placed in the wrong growth stage. Even if this spiracle is used an accuracy of over 90 per cent. can be maintained. If this is avoided, the accuracy of this quick method is over 97 per cent. and the speed with which the larvae can be examined is greatly increased. Whereas only 30 larvae could be dealt with in 8 hours when all the spiracles are examined, nearly 300 were placed in growth stage groups in the same length of time by the quicker method. Over 80 per cent. can be placed in the correct growth stage when the teeth on only one spiracle are counted; in less than 1 per cent. of larvae is it necessary to examine as many as five spiracles.

If the larvae are mounted in glycerine under a coverslip, the teeth on the spiracle are, in most cases, easily seen. In a few instances, however, owing to the dense contents of the larva, they can be distinguished only with great difficulty. In these cases they can be rendered more distinct by clearing the larva in cedarwood oil or similar medium, but this is seldom necessary.

The teeth on first growth stage larvae are not so distinct as those on the older larvae. Frequently there are no teeth at all on the sides of the smaller orifice; on others the tooth may appear only as a thickening of the peritreme at one point (fig. 2 b).

Further Analysis of August Square Yard Collection.

This quick method was used to determine the growth stages of the larvae from the August square yard collection which had not already been examined, so that all the 1,039 larvae were now divided into their eight groups, each one being a growth stage. It is interesting to compare this division with that based on total length measurements. In this latter division it was found that the larvae could be separated into four year-groups (fig. 1). In the first group, that is larvae from 2.01-3.6 mm., there were 335 larvae, or 32.24 per cent. of the population; in the second year-group, larvae from 3.61-7.2 mm., there were 324 larvae or 31.17 per cent.; in the third, larvae from 7.21-12.4 mm., there were 305 larvae or 29.31 per cent.; and in the fourth, larvae from 12.41-20.4 mm., there were only 75 larvae or 7.22 per cent. The eight growth stages contained 156, 188, 193, 160, 216, 73, 44, and 9 larvae respectively, or 15.02, 18.09, 18.58, 15.39, 20.79, 7.02, 4.24, and 0.85 per cent. of the population.

Some accounts of the life-history of wireworms claim that the larvae ecdyse twice each year (Balachowsky & Mesnil, 1935 ; Rymer Roberts, 1919 ; Subklew, 1934), although this is not universally accepted (Ford, 1917 ; Evans & Gough, 1942). If it is true that two moults occur each year, then larvae in their first and second growth stages would be in their first year, those in the third and fourth growth stages in their second year, and so on. If then the percentages of larvae in each of these pairs of growth stages are added, this will give the percentage of larvae placed in each year group by spiracle tooth numbers. In the first year-group there are 33.11 per cent. compared with 32.24 per cent. placed in this group by total length measurements ; in the second year group there are 33.97 per cent. compared with 31.17 per cent. ; in the third, 27.81 per cent. compared with 29.36 per cent. ; and in the fourth 5.09 per cent. compared with 7.22 per cent. Thus a division of the August square yard population based only on total length measurements allocated the larvae to year groups in nearly the same proportion as did one based on spiracle counts. But since a larva of any particular length may belong to any one of up to three growth stages, and hence to one or two different year groups, the individual larvae placed in the respective year groups by total length measurements will not necessarily be the same larvae as would be placed in these groups by counting the number of spiracle teeth.

Discussion.

One of the fundamental facts to be determined in any population study of a species is the relationship between size or instar on the one hand and age on the other. It has been seen that there is no fixed relationship between size and growth stage in the wireworm, *A. sputator*, and thus the relationship between size and age will also be uncertain. Any attempt to determine the relationship between instar and age is foiled by the interesting fact already noted of the ability of these wireworms to moult without growth, and the relationship between growth stage and age is as yet undetermined. This relationship can be investigated in either of two ways. The first is by rearing. In the past, it was necessary to rear the larva from egg to pupa. This has not been a very satisfactory method, partly owing to the great length of larval life. Now, however, it will be possible to shorten considerably the period during which each larva need be reared. If numbers of larvae in each of the different growth stages are reared for only a comparatively short length of time, information obtained from each of these will enable a complete picture of the life-history to be built up.

An alternative method of investigating the relationship between growth stage and age is by a study of data, similar to that given above for the larvae from the August collection, for a series of populations collected from one location at regular intervals of time over a period of one or more years. This method has the advantage that the larvae suffer no risk of being affected by abnormal conditions to which they may be subjected during rearing experiments. It is, perhaps, a more suitable method for the study of an insect with a long span of larval life, but the data it gives will not be so detailed and precise as those obtained by rearing.

One further problem presents itself. The number of growth stages through which a larva passes is not always constant. It would be interesting to know what effect this has on the length of larval life ; whether the length of larval life is the same for those individuals which have eight growth stages as it is for those which have only seven. If this is so, then the duration of each growth stage must vary with the individual larva. On the other hand, if the duration of each growth stage is constant, then larvae having seven growth stages will obviously have a shorter life than those with eight.

The phenomenon of ecdysis without growth has been noted in other insects. As long ago as 1883 Riley kept two specimens of *Trogoderma tarsale* Melsh. for 3½

years and collected from them 43 skins, but at the end of this time neither larva showed any appreciable increase in size. Wodsdalek (1912, 1917) also showed that this insect could go on moulting for several years without growing. In fact, the larvae became smaller, one even decreasing from the size of a fully grown larva (11 mm.) to that of a first-instar larva (1 mm.). Titschak (1926) found that the number of moults of *Tineola bisselliella* (Humm.) could be raised from 4 to 40 under certain conditions. In such cases there was little or no increase in size, while some larvae became smaller. Similar observations were made by Decker (1930, 1931) in *Luperina* and *Papaipema* and by Holloway, Haley & Loftin (1928) in *Diatraea saccharalis* (F.). In the last-named insect, the increased instar number was due to low temperatures, in *Luperina* it was due to "abnormal temperatures, bad food or other unfavourable conditions". In all other cases cited the moulting was induced by bad or inadequate food or by total starvation.

Titschak (1926) also showed that if after 2½ years of totally unsuitable feeding conditions the larvae were transplanted to suitable ones, they would then develop into normal adults. The same phenomenon was observed by Wodsdalek (1917) who induced a larva to increase and decrease in size alternately three times. It is not known whether after such a succession of non-growing instars wireworms will recover in the same way. None of these larvae completed its development to the pupal stage, and only one of them showed any increase in tooth number after having retained the same number of teeth during two or three moults.

It is unlikely that the moulting in these wireworms was caused by low temperatures, since they were reared at room temperature, and the moulting occurred in both summer and winter. It is possible that lack of nourishment caused them to ecdyse without growth. Unfortunately no record was kept of the amount of food given to these larvae, or of details of any other rearing conditions. It is not known at present whether this phenomenon in wireworms occurs under natural conditions or whether it is the result of the rearing technique.

The second fact which emerged from the examination of the cast skins of larvae reared in tubes was that some of the larvae pupated after eight growth stages, whilst others passed through only seven. These cast skins were then measured to see if there was a difference in the size of the larvae which had pupated in the different growth stages. This was not so; the parts of the prothorax and the last abdominal segment measured were almost the same size whether the larval skins were in the seventh or eighth growth stage.

Neither could the difference in growth stage be correlated with the size of the resulting adult. It seems, therefore, that the insect pupates after it has grown to a certain size, which is approximately the same for all larvae. This optimum size is reached by some wireworms in eight growth stages and by others in only seven.

In growth stage VII, then, there will be larvae of two sizes, those of the optimum size which are about to pupate, and those which have reached only penultimate size and will ecdyse into growth stage VIII before pupating. This was shown to be so by the usual measurements of the parts of the prothorax and last abdominal segments. The prothorax lengths of 12 larval skins in growth stage VII ranged from 0.54 mm. to 0.88 mm., of which four were between 0.54 mm. and 0.63 mm., and seven between 0.74 mm. and 0.88 mm. (one skin had the prothorax too torn to be measured). The last abdominal segments were from 1.09 mm. to 1.47 mm.; four were between 1.09 mm. and 1.26 mm. and eight between 1.33 mm. and 1.47 mm. Those skins which were in the group having the smaller prothorax also had the smaller last abdominal segment. This bimodality in measurements was also apparent in the skins from larvae in growth stage VI, and, less distinctly so, in growth stages V and IV. No skins in growth stages I to III were measured in this way.

The range in length of the prothorax of the larger larvae of growth stage VI was 0.60 mm. to 0.70 mm., and for the last abdominal segment 1.05 mm. to 1.19 mm. These measurements correspond roughly to the range in lengths found in the smaller larvae of growth stage VII, namely 0.54 mm. to 0.63 mm. for the prothorax, and 1.09 mm. to 1.26 mm. for the last abdominal segment. Similarly the larger larvae in growth stage V had prothorax lengths of 0.49 mm. to 0.53 mm., and last abdominal segment lengths from 0.84 mm. to 0.91 mm., compared with 0.49 mm. to 0.56 mm. and 0.81 mm. to 0.98 mm. respectively of the smaller larvae of growth stage VI.

The number of the larvae in these groups is very small, and it would be unwise to draw any conclusions from these measurements alone. However, other similar data are available, namely the total length measurement of each larva in the different growth stages found in the August square yard and the February square foot collections. For example, the range in the length of the 52 larvae of growth stage V of the February collection is from 6.41 mm. to 11.6 mm. Of these, 31 lie between 6.41 mm. and 8.4 mm. and 21 between 8.81 mm. and 11.6 mm. There are no larvae between 8.41 mm. and 8.8 mm. This tendency for the larvae in one growth stage to fall into two groups when their total length measurements are considered is more apparent in the February than in the August population. In the latter it is not evident at all in stage I, where more than 97 per cent. of the larvae are between 2.0 mm. and 2.8 mm. but it becomes increasingly apparent from stage II upwards.

When the two length measurement groups for any one growth stage are compared with those of the growth stage next above, it is seen that the range in length of the larger larvae of the first growth stage corresponds approximately with that of the smaller larvae of the succeeding one. For example in the February collection, larvae in growth stage III had total length measurements from 3.21 mm. to 6.4 mm., and formed two groups, 3.21 mm. to 4.4 mm. and 4.81 mm. to 6.4 mm. Growth stage IV had also two groups, one from 4.81 mm. to 6.4 mm. and the other from 6.41 mm. to 9.2 mm.

So far the difference in size of the larvae has been discussed only for those in growth stages I to VII. When larvae and cast skins in growth stage VIII were similarly examined, it was found that they also fell into two distinct size-groups. These facts suggest that a ninth growth stage may occur, and one cast skin has been found which provides additional evidence of this. The larva from which this exuvium was collected gave three successive cast skins. The spiracle tooth counts for the first two placed them in growth stages VII and VIII. The tooth numbers on the third skin were higher than those on either of the two others, and it was therefore thought that this must be a ninth growth stage larva. The average number of teeth on all the abdominal spiracles was 46.125, which lies within the limits, 42-62, of the thoracic range for group VIII (see p. 402); the thoracic spiracle tooth number average was 65. No other larvae or cast skins have yet been found with similar or higher counts.

After reaching this stage the larva had pupated. Measurements of the prothorax and last abdominal segment of the three skins gave surprising results. The prothorax length was 0.77 mm. in stage VII, 0.84 mm. in stage VIII, and 1.12 mm. in stage IX; the lengths of the last abdominal segment were 1.13 mm., 1.54 mm., and 1.75 mm. respectively for the three stages. Thus the larva when in its eighth growth stage was of the size in which larvae usually pupated, and yet it had ecdysed once more with increase of size and spiracle tooth number. It is possible that this abnormality was due to some character inherent in the larva itself. It may also have been caused by some environmental factor which prevented the larva from pupating, but not from growing, whilst in its eighth stage. Ripley (1923) found a similar occurrence in *Agrotis ypsilon* (Hfn.) where one larvae amongst 52 others had seven moults instead of six.

Further discussion on this one specimen would be unprofitable, and, moreover, would be a digression from the main subject of this section. It has been shown above that larvae of *Agriotes sputator* can be divided into two kinds—those which pupate after seven, and those which pupate after eight growth stages. The two sets of data—measurements of the cast skins and total length measurements of the larvae from the August and February soil samples—suggest that the division becomes apparent at an early stage in the life-history, and that the cause of this variation in growth stage number is one which is effective in the first instar or possibly earlier.

The variation in number of instars or growth stages through which a larva passes before it pupates is by no means uncommon in the Insecta, and the reasons for these variations are diverse. Rohwer & Middleton (1922), H. Miles (1931), Kreyenberg (1929), and Richards & Thomson (1932) found that it varied with sex, the female usually having more moults than the male. Increased temperature may also cause an increase in the number of moults (Gierke 1932, Kreyenberg 1929), or a decrease in number (Klein 1932, Parker 1930), whilst poor inadequate supplies of food usually lengthen the life-cycle, thereby increasing the number of instars (Stone 1941, Back & Cotton 1926, Potter 1935, Gaines & Campbell 1935, Decker 1930, 1931).

In *Agriotes sputator*, sex does not appear to be the cause of variation in the number of growth stages. Of the 36 larvae which had pupated, 14 developed into complete adults. Of eighth growth stage larvae four were males and seven females, and of seventh growth stage, one was a male and two were females.

Temperature differences which occur during the last part of the larval life of these wireworms do not effect the growth stage in which the larva pupates. The larvae which had pupated in the laboratory, and from which the cast skins were collected, were reared at constant temperatures of 15, 20 and 25 °C. Some of the larvae which had been reared and had pupated at the same temperature had passed through eight and the remainder only seven growth stages.

Since only large larvae were reared until they pupated, no information is available about the amount of food with which they were supplied in their early stages. They were, however, all collected from the same locality, an area of permanent grassland, so that they should all have had equal opportunities of obtaining an adequate supply of food.

In other instances the increase or decrease in the number of moults is accompanied by a larger or smaller final stage larva. The phenomenon displayed by larvae of *A. sputator* of reaching the same ultimate size in a varying number of growth stages has been found in some other insects. Horsfall (1941) observed it in *Epicauta*, but in this instance the larvae, whether they were going to pupate after five or seven moults, were the same size in the first five instars. In other cases, as in *A. sputator*, the difference in size of those larvae which will pupate after a different number of growth stages increases after the first moult, although this difference is not always clearly seen until the later instars.

The difference in number of instars in the above type of insect may also be due to sex (H. Miles, 1931), or to insufficient food (Decker, 1930, 1931; Gaines & Campbell, 1935). It has already been seen that neither of these factors will account for the difference in number of growth stages in *A. sputator*. Other authors (Satterthwait, 1933; Waloff, 1948; Good, 1933; Titschak, 1926) found that the difference in instar number was due to individual differences in the larvae, and not to any environmental conditions. In the last two cases the variation was attributed to inherited characters.

The observations described here on *A. sputator* seem most nearly to parallel those made by M. Miles (1933) on *Plodia interpunctella* (Hb.). She found that the number of larval instars varied from five to seven and was independent of environment.

Measurements of the width of the head capsule had been taken to indicate the amount of growth. These measurements were fairly uniform for larvae in the first instar, the variation increasing from the second to fourth, and although less so, was still considerable in the fifth and sixth instars. She concludes that "the number of larval stadia was associated with the rate of growth at each ecdysis, larvae growing rapidly at each ecdysis having fewer stadia than those growing more slowly", and that "variation in the number of larval stadia, rate of larval growth and duration of larval life are the result of acceleration or retardation of physiological processes".

That the number of growth stages in *A. sputator* is also influenced by the amount of growth at each ecdysis is obvious, and that it is the result of acceleration or retardation of physiological processes follows naturally. But what is the cause of this acceleration or retardation? Since the influence shows its effects after the first moult, it is in the early stages that the cause should be sought. It is possible that environmental factors influencing the amount of growth in the first instar would result in two different sized second instar larvae. The differences between these would become more marked as the larvae grew, resulting in some larvae pupating at an optimum size after seven growth stages, and others at the same size after eight. But larvae living in a locality such as that from which these were obtained would have the same chance of food, and live under the same physical conditions unless they were hatched at different times of the year. Hatching in wireworms extends over a period of just over one month, and according to Rymer Roberts (1919) the larvae all moult at nearly the same time in the summer. It would be possible for earlier hatched larvae, having had a longer feeding period, to grow more at the first ecdysis than later hatched ones.

It is also possible that the difference may be due to the size of the egg and the amount of food available to the developing larva. If this were so, the difference in size should be apparent in the first growth stage larva. As has been seen, in the first growth stage larvae from the August collection the larvae are not divided into two size-groups. The range of length of the larvae in this group is so small, that it is possible that any difference in size may be obscured by the coarse unit of measurement. More rigorous investigations will have to be made before this point can be finally settled.

It may be, however, that in *Agriotes sputator*, as in *Agrotis* (Ripley, 1923), *Tribolium* (Good, 1933) and *Tineola* (Titschak, 1926), this variation in growth stage number is not due to any environmental factor, but to some inherited character. Those larvae which pupate after seven growth stages and those which pupate after eight, may represent two different strains.

There are, then, the following possible causes—the size of the egg, hence the amount of food available before hatching; the time of the year at which hatching takes place, hence the amount of food available and the prevailing temperature whilst the larva is in the first growth stage; or heredity. To which of these factors this phenomenon should be attributed cannot be decided until further investigations have been made.

Summary.

The different instars of *Agriotes sputator* larvae cannot be distinguished by measurements of total length or of various parts of the wireworm.

The number of teeth on the mesothoracic and abdominal spiracles increases with age, and an examination of 700 wireworms showed that the average numbers of teeth on the two thoracic or on all the abdominal spiracles fell into eight groups. These criteria were valid for populations collected at different times of the year.

The number of teeth on the thoracic spiracles of a larva in any particular group approximates to that on the abdominal spiracles of a larva in the next larger group.

The eight groups formed by counting either the thoracic or the abdominal spiracle teeth represent *growth stages* and not necessarily instars.

The larvae may sometimes moult without growth, a phenomenon probably caused by an inadequate supply of food. At such an ecdysis the number of spiracle teeth does not increase.

The larvae pupate after attaining an optimum size, and reach this size in seven or eight growth stages.

A quick method of determining the growth stage to which a larva belongs is given, whereby more than 80 per cent. of the larvae are placed in their correct growth stages by counting the teeth on one spiracle only; for less than 1 per cent. of the larvae is it necessary to examine as many as five spiracles; an accuracy of more than 97 per cent. can be maintained.

The possibility of determining the relationship of growth stage and age is discussed.

The division of the larvae into those which will pupate after seven, and those which will pupate after eight growth stages is apparent at a very early stage in the life history. One larva was found which appeared to be in its ninth growth stage.

The cause of this difference in growth stage number is unknown. It may be due to the size of the egg, to the time of the year at which hatching occurs, or to heredity.

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References.

- BACK, E. A. & COTTON, R. T. (1926). The Cadelle.—Dep. Bull. U.S. Dep. Agric., no. 1428, 41 pp.
- BALACHOWSKY, A. & MESNIL, L. (1935). Les insectes nuisibles aux plantes cultivées, **1**, pp. 754–764. Paris.
- DECKER, G. C. (1930). The biology of the Four-lined Borer, *Luperina stipata* (Moir.).—Res. Bull. Iowa agric. Exp. Sta., no. 125, pp. 125–164.
- DECKER, G. C. (1931). The biology of the Stalk Borer *Papaipema nebris* (Gn.).—Res. Bull. Iowa agric. Exp. Sta., no. 143, pp. 291–351.
- EVANS, A. C. & GOUGH, H. C. (1942). Observations on some factors influencing growth in wireworms of the genus *Agriotes* Esch.—Ann. appl. Biol., **29**, pp. 168–175.
- FORD, G. H. (1917). Observations on the larval and pupal stages of *Agriotes obscurus* Linn.—Ann. appl. Biol., **3**, p. 97.
- GAINES, J. C. & CAMPBELL, F. L. (1935). Dyar's rule as related to the number of instars of the Corn Ear Worm, *Heliothis obsoleta* (Fab.) collected in the field.—Ann. ent. Soc. Amer., **28**, pp. 445–461.

- VON GIERKE, E. (1932). Über die Häutungen und die Entwicklungsgeschwindigkeit der Larven der Mehlmotte, *Ephestia kühniella* Zeller.—Arch. EntwMech. Org., **127**, pp. 387–410.
- GOOD, N. (1933). Biology of the flour beetles, *Tribolium confusum* Duv. and *T. ferrugineum* Fab.—J. agric. Res., **46**, pp. 327–334.
- GUÉNIAT, E. (1934). Contribution à l'étude du développement et de la morphologie de quelques Elatérides (Coléoptères).—Mitt. schweiz. ent. Ges., **16**, pp. 167–298.
- HOLLOWAY, T. E., HALEY, W. E. & LOFTIN, J. C. (1928). The Sugar-cane Moth Borer in the United States.—Tech. Bull. U.S. Dep. Agric., no. **41**, 76 pp.
- HORSEFALL, W. R. (1941). Biology of the Black Blister Beetle (Coleoptera : Meloidae).—Ann. ent. Soc. Amer., **34**, pp. 114–126.
- KLEIN, H. Z. (1932). Der Einfluss der Temperatur und Luftfeuchtigkeit auf Entwicklung und Mortalität von *Pieris brassicae* L.—Z. angew. Ent., **19**, pp. 395–448.
- KREYENBERG, J. (1929). Experimentell-biologische Untersuchungen über *Dermestes lardarius* L. und *Dermestes vulpinus* F.—Z. angew. Ent., **14**, pp. 140–188.
- MILES, H. W. (1931). Growth in the larvae of Tenthredinidae.—J. exp. Biol., **8**, pp. 355–364.
- MILES, M. (1933). Observations on growth in larvae of *Plodia interpunctella* (Hübner).—Ann. appl. Biol., **20**, pp. 297–307.
- PARKER, J. R. (1930). Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Sauss. and *Cannula pellucida* Scudder (Orthoptera).—Bull. Mont. agric. Exp. Sta., no. **223**, 132 pp.
- POTTER, C. (1935). The biology and distribution of *Rhizopertha dominica* (Fab.).—Trans. R. ent. Soc. Lond., **83**, pp. 449–574.
- RICHARDS, O. W. & THOMSON, M. A. (1932). A contribution to the study of the genera *Ephestia*, Gn. (including *Strymax* Dyar) and *Plodia*, Gn. (Lepidoptera, Phycitidae), with notes on parasites of the larvae.—Trans. ent. Soc. Lond., **80**, pp. 169–250.
- RILEY, C. V. (1883). Number of moults and length of larval life as influenced by food.—Amer. Nat., **17**, pp. 547–548.
- RIPLEY, L. B. (1923). The external morphology and post-embryology of Noctuid larvae.—Ill. Biol. Monogr., **8**, no. **4**, 102 pp.
- ROHWER, S. A. & MIDDLETON, W. (1922). North American sawflies of the sub-family Cladinae.—Proc. U.S. nat. Mus., **60**, pp. 1–46.
- RYMER ROBERTS, A. W. (1919). On the life history of "wireworms" of the genus *Agriotes* Esch. with some notes on that of *Athous haemorrhoidalis* F. I.—Ann. appl. Biol., **6**, pp. 116–135.
- RYMER ROBERTS, A. W. (1921). On the life history of "wireworms" of the genus *Agriotes* Esch. with some notes on that of *Athous haemorrhoidalis* F. II.—Ann. appl. Biol., **8**, pp. 193–215.
- RYMER ROBERTS, A. W. (1922). On the life history of "wireworms" of the genus *Agriotes* Esch. with some notes on that of *Athous haemorrhoidalis* F. III.—Ann. appl. Biol., **9**, pp. 306–324.
- SALT, G. & HOLICK, F. S. J. (1944). Studies of wireworm populations. I. A census of wireworms in pasture.—Ann. appl. Biol., **31**, pp. 52–64.
- SATTERTHWAIT, A. F. (1933). Larval instars and feeding of the Black Cutworm, *Agrotis ypsilon* Roh.—J. agric. Res., **46**, pp. 517–530.

- STONE, M. W. (1941). Life history of the Sugar Beet Wireworm in Southern California.—Tech. Bull. U.S. Dep. Agric., no. 744, 87 pp.
- SUBKLEW, W. (1934). *Agriotes lineatus* L. und *Agriotes obscurus* L. (Ein Beitrag zu ihrer Morphologie und Biologie).—Z. angew. Ent., **21**, pp. 96–122.
- TITSCHAK, E. (1926). Untersuchungen über das Wachstum, den Nahrungsverbrauch und die Eierzeugung. II. *Tineola bisselliella* Hum. Gleichzeitig ein Beitrag zur Klärung der Insektenhäutung.—Z. wiss. Zool., **128**, pp. 509–569.
- WALOFF, N. (1948). Development of *Ephestia clutella*, Hb. (Lep., Phycitidae) on some natural foods.—Bull. ent. Res., **39**, pp. 117–130.
- WODSEDALEK, J. E. (1912). Life history and habits of *Trogoderma tarsale* (Melsh.), a museum pest.—Ann. ent. Soc. Amer., **5**, pp. 367–382.
- WODSEDALEK, J. E. (1917). Five years of starvation of larvae.—Science, (N.S.) **46**, pp. 366–367.

A CHECK-LIST AND HOST-LIST OF IXODOIDEA FOUND IN NYASALAND,* WITH DESCRIPTIONS AND BIOLOGICAL NOTES ON SOME OF THE RHIPICEPHALIDS.

By S. G. WILSON, Ph.D., M.R.C.V.S.

The geographical distribution in the African Continent of very few of the species of Ixodoidea has been fully determined. *Rhipicephalus sanguineus* (Latr.) and certain species of *Boophilus* are known to be widely distributed, while others, such as *R. duttoni* Neum., have been recorded from only one or two localities. The present study was primarily undertaken to determine the genera and species of ticks occurring in Nyasaland, but it is hoped it will contribute to the wider problems of the distribution in Africa of the several species.

Weekly collections of cattle ticks in the Central and Northern Provinces of Nyasaland were made systematically for three years, but collection from game, wild carnivora and rodents were more fortuitous. Live ticks were placed, as soon as collected, in a killing fluid, the most successful fluid being that recommended by Boardman (1944), and later transferred to a formalin-chloroform preserving solution. Attempts were made to rear every species collected and *R. appendiculatus* Neum., *R. sanguineus*, *Amblyomma variegatum* (F.) and *Boophilus* spp. were bred without difficulty. The breeding of ticks in many cases assisted in identification. Thus, engorged living nymphs collected from a cane-rat were identified with more certainty after moulting as adult *R. simpsoni*. Larvae of many species were similarly treated with equal success.

The climate and vegetation of the area where most of the ticks were collected has already been described (Wilson, 1946).

Check-List and Host-List of Species Recorded.

Seven genera of the family IXODIDAE and three of the family ARGASIDAE were recorded in the present survey. The species identified, together with the hosts on which they were found, are given below. The recent revision of the genus *Rhipicephalus* by Zumpt (1942-43) has been considered but his conclusions do not always agree with the study of the present Nyasaland material and the species *R. neavei* Warb. and its variety *punctatus* Warb. have been retained. The nomenclature of the *Boophilus* and *Hyalomma* genera is that proposed by Minning (1934) Schulze (1930) and Schulze and Schlottke (1929) respectively. Cooley and Kohls (1944) have recently studied the ARGASIDAE occurring in N. America and have re-established the genera *Ornithodoros* and *Otobius* in addition to the genus *Argas* and this classification is followed in view of the differences clearly demonstrated by the three Nyasaland species.

*This article is based on Part I of a Thesis submitted to the University of Edinburgh for the Degree of Doctor of Philosophy.

FAMILY IXODIDAE

Genus and Species	Hosts
<i>Rhipicephalus appendiculatus</i> Neum.	Cattle, sheep, goat, donkey, dog, cane-rat (<i>Aulacodus</i>) (N).
„ <i>neavei</i> Warb.	Roan antelope, eland, kudu, bush-buck, gwape, duiker, wart-hog, <i>Lepus</i> spp., cattle, sheep, buffalo.
„ <i>neavei</i> var. <i>punctatus</i> Warb.	Kudu, impala, reed-buck, hartebeest, gwape, <i>Lepus whytei</i> , buffalo, cattle.
„ <i>supertritus</i> Neum.	Sable antelope, buffalo, hartebeest.
„ <i>sculptus</i> Warb.	Roan antelope, zebra.
„ <i>masseyi</i> Nutt. & Warb.	Bush-buck, nyala, buffalo, dog.
„ <i>simus</i> Koch	Cattle, buffalo, leopard, jackal, cheetah, lion, dog, ant-bear, wart-hog, and <i>Rattus</i> sp. (N).
„ <i>tricuspis</i> Dön.	Cattle, sheep, goat, dog, buffalo, lion, duiker, gwape, steinbuck, reed-buck, wart-hog, serval and <i>Lepus</i> sp.
„ <i>simpsoni</i> Nutt.	Cane-rat.
„ <i>falcatus</i> Neum.	Roan antelope, buffalo, horse, <i>Phacochaerus aethiopicus</i> .
„ <i>capensis</i> Koch	Cattle, goat, pig, wart-hog, reed-buck and civet.
„ <i>ayrei</i> Lewis	Buffalo, lion, cheetah.
„ <i>sanguineus</i> (Latr.)	Dog, leopard, hare (<i>Lepus whytei</i>), lion, ant-bear, gwape, kudu, jackal, cheetah, hedgehog, cattle, sheep, goats.
<i>Boophilus</i> (<i>Uroboophilus</i>) <i>fallax</i> Minning	} Cattle, sheep, goats.
„ (<i>Palpobophilus</i>) <i>decoloratus</i> (Koch)	
<i>Amblyomma variegatum</i> (F.)	Cattle, sheep, goats, reed-buck, sable antelope, duiker, gwape, buffalo, dog, poultry (N), <i>Lepus whytei</i> (N), hedgehog (N), cane-rat (N), cheetah (N), man (L).
„ <i>tholloni</i> Neum.	Elephant.
„ <i>petersi</i> Karsch	Rhinoceros.
„ <i>marmoreum</i> Koch	Tortoise.
<i>Hyalomma impressum</i> Koch	Cattle, sheep, eland, buffalo, lion, ant-bear, <i>Lepus whytei</i> (N).
<i>Aponomma falsolaeye</i> Schulze... ..	Snake (sp.?).
„ <i>exornatum</i> (Koch)	Iguana.
<i>Ixodes pilosus</i> Koch	Cattle, stein-buck, gwape, duiker, reed-buck, lion, squirrel, dog, serval.
<i>Haemaphysalis leachi</i> (Aud.)	Dog, leopard, civet, cheetah, jackal, mongoose, hedgehog, lion, squirrel, porcupine, <i>Rattus</i> sp. (N).
„ <i>hoodi</i> Warb. & Nutt.	Partridge.

FAMILY ARGASIDAE

<i>Argas persicus</i> (Oken)	Poultry.
<i>Otobius magnini</i> (Dugès)	Horse.
<i>Ornithodoros moubata</i> (Murr.)	Native huts, domestic pigs.

(N)—Nymphs only collected on host.

(L)—Larvae only collected on host.

Descriptive Notes on some of the Rhipicephalids.

The members of the genus *Rhipicephalus* are arranged into the four following arbitrary groups: I "*appendiculatus*", II "*simus*", III "*capensis*" and IV "*sanguineus*".

I—"Appendiculatus" Group.

At least eight species in this group occur in East or Central Africa, i.e., *R. appendiculatus*, *R. neavei*, *R. neavei* var. *punctatus*, *R. supertritus*, *R. sculptus*, *R. kochi* Dön., *R. duttoni* Neum. and *R. masseyi*. The West African species, *R. ziemannii* Neum. and the recently described species *R. muhlensi* Zumpt (1943), would also fall within this group. All the ticks in this group show numerous medium and

fine punctuations universally distributed over the scutum, or certain lateral areas may remain smooth and glossy. There may be some scutal ridging or sculpture as in *R. sculptus* and to a less extent in *R. supertritus* and *R. appendiculatus*. The basis capituli is broader than long with the lateral angles anterior and obtuse, except in *R. neavei* where they are acute and re-curved. The projection on coxa I is visible dorsally and the median and posterior lateral dorsal grooves are well developed. The adanal plates show considerable variation and therefore assist in differentiating the various species.

***Rhipicephalus neavei* Warburton (1912) (fig. 1).**

This is a very distinct species easily distinguished from *R. appendiculatus* and *R. neavei* var. *punctatus* and the Nyasaland material conforms very closely to the description given by Warburton (1912). In the male, the lateral angles of the basis capituli are acute and strongly re-curved especially when viewed ventrally. The anterior process of coxa I is prominent dorsally. Medium-sized punctations, better defined than in *R. appendiculatus* are found all over the scutum except on the region immediately in front of the eyes. The cervical grooves are deep oval pits continued

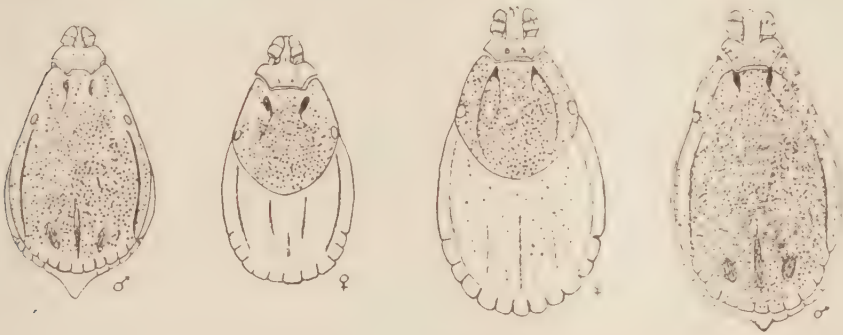


Fig. 1.—*Rhipicephalus neavei*, dorsum of engorged male and female.

Fig. 2.—*Rhipicephalus neavei* var. *punctatus*, dorsum of engorged female and male.

posteriorly as divergent shallow linear depressions. The lateral grooves and the three posterior dorsal furrows are well defined. Eyes large, yellowish, and flat, with a few large punctations near the mesial border. The body may extend beyond the scutum and bear plaques. The adanal plates are as shown in fig. 10 b.

In the female the lateral angles of the basis capituli are acute and re-curved as in the male, while the lateral cervical grooves of the scutum are absent or indefinite and the marginal scutal area is raised, convex, glossy and almost devoid of punctations. The central area of the scutum is closely punctated with medium-sized punctations with a few also on the scapulae. Finer punctations also occur on the median anterior cervical field and on the smooth lateral areas.

***R. neavei* var. *punctatus* Warburton (1912) (fig. 2).**

This variety is difficult to define as many specimens approach *R. appendiculatus* in the structure of the basis capituli, while others resemble *R. capensis* in the density of the punctation. The scutum of the female has a superficial resemblance to *R. sanguineus*. It bears little resemblance to *R. neavei*.

Male.—The angles of the basis capituli are less acute and less re-curved than those of *R. neavei*, the cornuae are short and blunt, and the dorsal surface is punctated

especially near the posterior border and many carry a row of hairs as in *R. appendiculatus*. Article I of the palps is clearly visible dorsally and articles II and III are equal and broader than long, article III having a blunt rounded anterior margin and a concave dorsal surface. The punctations on the scutum are coarse, numerous and deep, but a few finer punctations also occur on the anterior cervical and scapular regions. The anterior cervical grooves resemble those of *R. neavei*, and the lateral grooves and the three posterior dorsal grooves are also distinct. The yellowish body protrudes in engorged specimens laterally and posteriorly beyond the scutum. The anal plates have rounded obtuse posterior external angles and an internal protuberance on the median border distally, similar to that found in *R. neavei* is common. Accessory plates (fig. 10 c, d) are strongly developed in some specimens (cf. Warburton, 1912).

Female.—The basis capituli is twice as broad as long, with the lateral angles median and acute. The palps resemble those of *R. appendiculatus*. The scutum is oval, longer than broad and deeply punctated, with a few finer punctations in the anterior regions. The anterior portions of the cervical grooves are deep and converging and continued posteriorly by faintly delineated diverging furrows. The lateral groove is well marked, reaches the posterior border, is deeply punctated, and defines the raised lateral areas from the central depressed scutal area. The eyes are salient and placed far back on the lateral border and are limited mesially, as in the male, by an adjacent row of punctations.

***Rhipicephalus supertritus* Neumann (1907) (figs. 3 and 4).**

R. coriaceus Nuttall & Warburton 1908, Proc. phil. Soc. Camb., **14**, p. 402.

R. supertritus was only collected on three occasions during the present survey. It is larger than *R. appendiculatus* with the scutum more coarsely punctated and with reticulations rather than punctations on the surfaces of the cervical and posterior grooves.

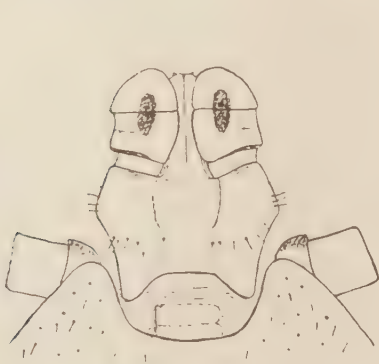


Fig. 3.—*Rhipicephalus supertritus*, dorsal aspect of capitulum.

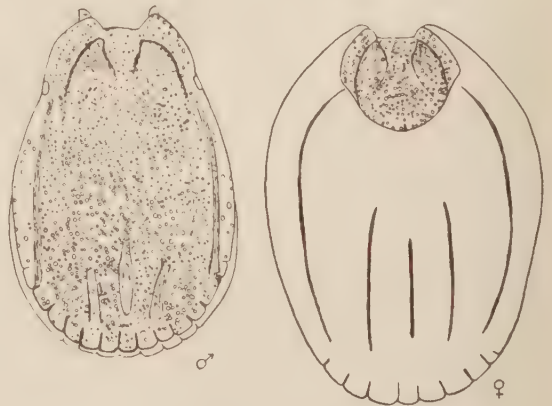


Fig. 4.—*Rhipicephalus supertritus*, dorsal aspect of scuta of partially engorged male and engorged female.

Male.—The basis capituli differs little from *R. appendiculatus*. Article II of the palps is larger than article III and both are concave dorsally. The projection on coxa I is visible dorsally. Scutal punctations are numerous, medium-sized and tend to form rugosities on the posterior part of the scutum. The festoons are distinct and

in engorged specimens the protruding abdomen carries plaques and three finger-like protrusions. The anal plates (fig. 10 *e*) resemble those of *R. appendiculatus*.

Female differs from *R. appendiculatus* in having more coarse dense scutal punctations which may be confluent in the central posterior region to give a rugose appearance to the scutum. The lateral grooves are pronounced and reach the posterior margin with definite reticulate surfaces on the cervical depressions and a reticulated band extends backwards median to the lateral groove. A fuller description of this species is given by Theiler (1947) from material collected during the present survey in Nyasaland.

***Rhipicephalus sculptus* Warburton (1912) (figs. 5, 6 and 7).**

This rare species was only collected on two occasions during the entire survey.

Male.—The basis capituli is relatively large with the lateral angles anterior and obtuse and the dorsal surface punctated and carrying a row of hairs. Article II of the palps is rectangular and article III ends bluntly and the dorsal surfaces of both articles are concave. The projection on coxa I is visible dorsally. The cervical grooves on the scutum are well defined and the cervical depressions extend backwards to meet a semi-circular raised glossy ridge which runs, about midway, across the scutum.

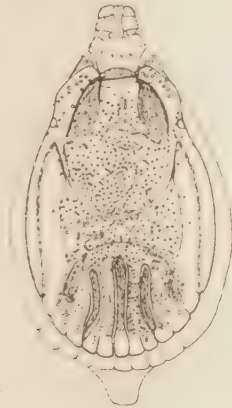


Fig. 5.—*Rhipicephalus sculptus*, dorsum of engorged male.

The three posterior dorsal furrows are limited laterally and anteriorly by narrow glossy ridges. The surface of the cervical depression and posterior dorsal furrows are reticulated, and reticulated depressions also usually occur external to the lateral posterior dorsal furrows. Medium-sized scutal punctations are numerous, but a group of very large punctations occur on the scapulae while very fine punctations occur on the anterior median cervical area and on the raised lateral borders. The anal plates (fig. 10 *f, g*) show some variation. Some conformed to the description given by Warburton (1912), but on most of the Nyasaland material and on many of the type species in the British Museum, a posteriorly directed spur is formed at the junction of the posterior and lateral borders while a similar inwardly directed spur is found at the junction of the posterior and median borders.

Female.—Distinguishing features are found on the scutum which is circular, with well-developed lateral grooves reaching the posterior margin and defining broad raised lateral borders. The surfaces of the anterior depressed areas are reticulated and a band of reticulations runs posteriorly internal and adjacent to the lateral grooves. Punctations in the central field are medium-sized, discreet, and uniformly

distributed but a group of large punctations occurs on the lateral border while small ones also occur on the lateral border, anterior to the eyes and on the anterior median cervical field. The eyes are medium sized and bordered mesially by a groove which would appear to represent several coalesced punctations. The body is fringed dorsally by a row of stout white hairs and a further row of hairs is found along the marginal furrow.



Fig. 6.—*Rhipicephalus sculptus*, dorsal aspect of capitulum and scutum of engorged female.



Fig. 7.—*Rhipicephalus sculptus*, dorsal aspect of capitulum and anterior portion of scutum of male.

***Rhipicephalus masseyi* Nuttall (1908) (figs. 8 and 9).**

R. attenuatus Neumann (1908), Arch. Parasitol., 12, p. 12.

R. masseyi is a medium-sized tick with the scutum reddish-brown to yellowish-brown in colour. It was collected on only one occasion from a dog but type specimens and other material were examined at the British Museum.

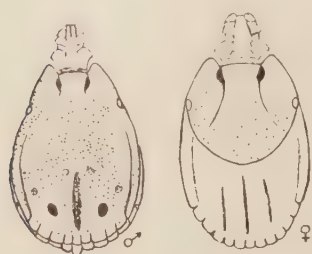


Fig. 8.—*Rhipicephalus masseyi*, dorsal aspect of engorged male and female.



Fig. 9.—*Rhipicephalus masseyi*, A. Capitulum of female; B. Capitulum of male. (Dorsal aspects.)

Male.—The basis capituli is broader than long with obtuse anterior lateral angles and a row of hairs running transversely across the punctated dorsal surface as in *R. appendiculatus*. Article I of the palps is visible dorsally and article II and III are equal, broader than long and concave dorsally. A row of hairs projects from the convex lateral border of the palps. The cervical grooves on the scutum are small deep crescentic pits continued posteriorly by very shallow indistinct divergent depressions. The lateral grooves are indicated anteriorly by shallow punctations which become deeper posteriorly and form definite grooves. Zumpt (1943) records these lateral grooves as missing. In all the specimens examined only the median and one pair of postero-lateral dorsal furrows are present, the latter being oval, though Zumpt (1943) states that in some specimens they are long and linear and approach those which he describes for *R. aurantiacus*. Two pairs of foveolae are also present, placed diagonally, anterior to the postero-lateral grooves, the median pair being anterior. Fine punctations are numerous, some specimens showing punctations similar to the very fine punctations seen in *R. simus*. Occasional large punctations also occur. The adanal plates are broad posteriorly with rounded external and internal angles and with an inner projection on the internal border anterior to the internal angle. Small triangular accessory plates are present (fig. 10 h).

Female.—The basis capituli is broader than in the male with the lateral angles not very acute; cornua short and bluntly rounded. The palps are well developed with article I visible dorsally. The scutum is circular in some specimens but more elongated in others. Cervical grooves as in the male but the lateral grooves are absent. Medium-sized punctations are numerous in the central field but scarce on the raised lateral areas.

II—"Simus" Group.

The "*simus*" group is represented in Nyasaland by four species: *R. simus*, *R. tricuspis*, *R. simpsoni* and *R. falcatus*. Two distinct types of scutal punctations are present on all four species: coarse punctations which are relatively sparse and tend to be arranged in four longitudinal rows, and fine punctations which may be "pin-prick" like in size and are usually numerous and uniformly scattered over the scutum. The median and postero-dorsal grooves are not well developed. *R. distinctus* Bedford, *R. longicoxatus* Neumann and *R. complanatus* Neumann were not recorded from Nyasaland.

This group has recently been revised by Zumpt (1943) who gives a description of seven African species including one new one, *R. reichenowi*, and one new sub-species *R. simus longoides*. The latter is the West African form of *R. simus simus* and the males are differentiated by the shape of the anal plates and number of median festoons that protrude. The females are indistinguishable and Zumpt postulates that populations exist where, owing to the variability of the shape of the anal plates, it would be impossible to distinguish the sub-species from the "*nominat form*".

From a study of their morphology and seasonal incidence in Nyasaland, *R. simus* Koch and *R. tricuspis* Dön. are regarded as two distinct species. A recent description of these two ticks has been given by Theiler (1947).

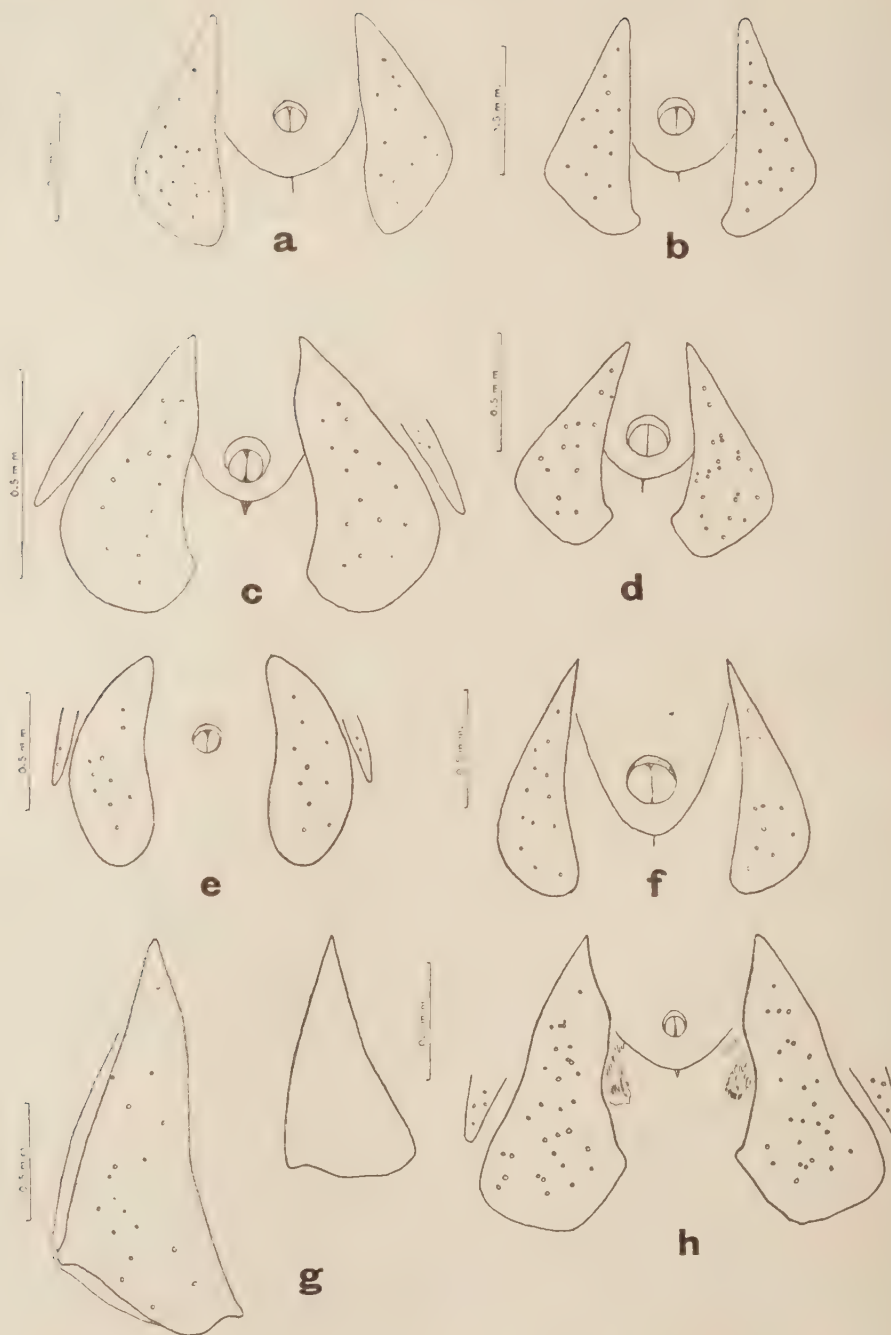


Fig. 10.—"Appendiculatus" group—anal plates of (a) *R. appendiculatus*; (b) *R. neavei*; (c) *R. neavei* var. *punctatus* (Nyasaland); (d) *R. neavei* var. *punctatus* (British Museum specimen); (e) *R. supertritus*; (f) *R. sculptus* (type specimen British Museum); (g) *R. sculptus* (type specimen British Museum and Nyasaland); (h) *R. masseyi*.

***Rhipicephalus simpsoni* Nuttall (1910) (fig. 11).**

This species was only collected from one host, the large cane-rat, and does not feed on domestic animals. Apart from this, the males may be distinguished from *R. simus* and *R. tricuspis* by the sparse coarse scutal punctations while the fine punctations are numerous and medium sized as in *R. tricuspis*. The lateral angles of the basis capituli are median in position and are acute or re-curved. The scutum

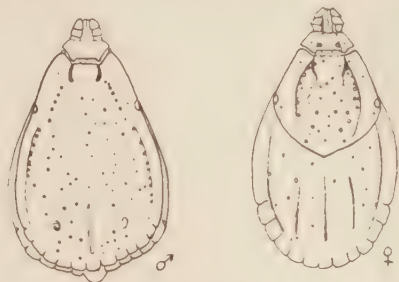


Fig. 11.—*Rhipicephalus simpsoni*, dorsal aspect of engorged male and female.

is reddish-brown with coxa I visible dorsally, and with cervical grooves very similar to those of *R. simus*. The posterior dorsal depressions, especially the elongated median depression, are clearly defined. In the female, the scutum is longer than broad and the punctations are generally confined to the central field and the raised glossy lateral margins may be unpunctated. The large punctations are irregular and few, with more numerous smaller punctations in the central field. The posterior border of the scutum is uniformly convex except for a slight median protrusion. The lateral grooves are not so distinct as in either *R. simus* or *R. tricuspis*.

***Rhipicephalus falcatus* Neumann (1908) (figs. 12 and 13).**

In this species the coarse punctations on the scutum of the male are few and irregularly scattered while small punctations are numerous. The lateral angle of the

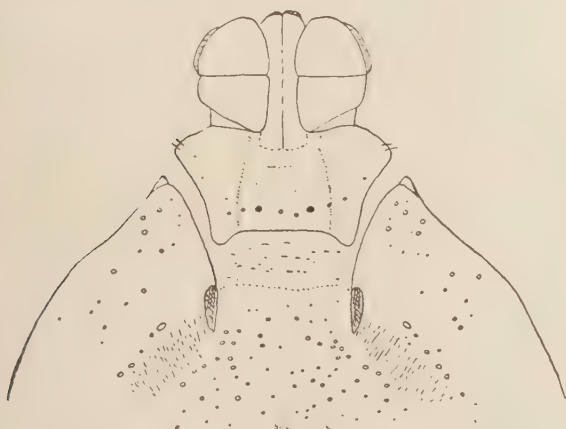


Fig. 12.—*Rhipicephalus falcatus*, dorsal aspect of capitulum and anterior portion of scutum of male.

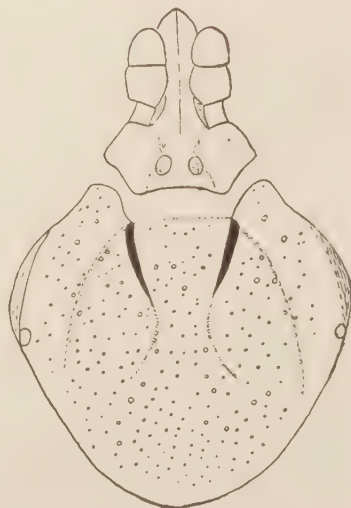


Fig. 13.—*Rhipicephalus falcatus*, dorsal aspect of capitulum and scutum of female.

basis is anterior and obtuse. The anterior processes of coxa I are only slightly visible dorsally. The cervical grooves resemble *R. simus*. The lateral groove is well marked, punctated and includes two festoons. The posterior dorsal furrows are only slightly indicated. The body protrudes beyond the scutum and the festoons carry plaques. The median and two adjacent festoons are especially large forming three protrusions in engorged specimens. In some of the specimens examined at the London School of Hygiene and Tropical Medicine, the two lateral festoons may be enlarged giving a resemblance to *R. ayrei*. The adanal plates are sickle shaped with accessory plates well developed (fig. 14 b).

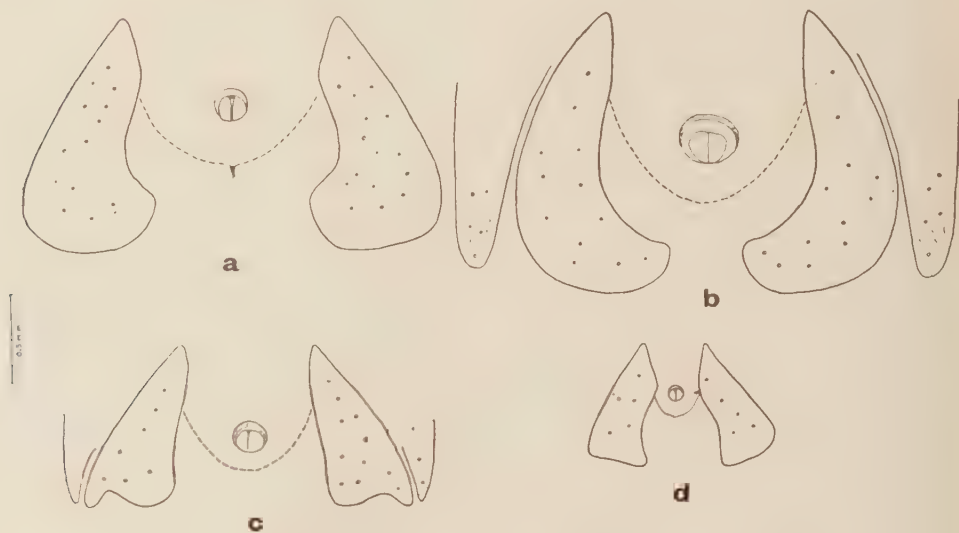


Fig. 14.—“*simus*” group—anal plates of (a) *R. simus*; (b) *R. falcatus*; (c) *R. tricuspis*; (d) *R. simpsoni*.

The basis capituli of the female is twice as broad as long with lateral angle median and acute. The palps resemble those of *R. tricuspis*. The scutum is as broad as long, with deep cervical grooves and the lateral grooves are well marked reaching the posterior border of the scutum and define raised lateral borders. Medium-sized punctations are numerous but large punctations are few and irregularly distributed. The convex lateral border of the scutum is bent ventrally.

III—“*Capensis*” Group.

Only two Nyasaland species, *R. capensis* Koch and *R. ayrei* Lewis belong to this group and considerable difficulty was experienced in differentiating females and unengorged males (figs. 15 and 16). Both are large species with reddish-brown to dark-brown scuta and reddish legs. The basis capituli is about twice as broad as long with prominent lateral angles but in *R. ayrei* the antero-lateral border is interrupted by a collar-like groove. The scutal punctations in both species are medium sized, densely packed, deep, and uniformly distributed over the scutum. The lateral grooves are well marked and include two festoons in both species. The posterior dorsal grooves are usually faintly marked but show some variation. In *R. capensis*, the lateral posterior grooves are oval but are usually comma-shaped in *R. ayrei*.



Fig. 15.—A. *Rhipicephalus capensis*, dorsal aspect of engorged male. B. *Rhipicephalus ayrei*, dorsal aspect of engorged male.

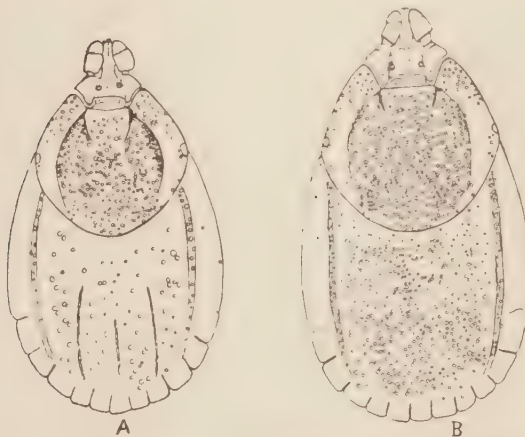


Fig. 16.—Dorsal aspect of females : A. *Rhipicephalus capensis*, B. *Rhipicephalus ayrei*.

The adanal plates cannot be used to distinguish the two species as they show considerable variation (fig. 17). The most conspicuous difference is shown by the engorged *R. ayrei* females where protuberances of the body contour occur opposite the two lateral festoons while the median festoon also protrudes as a dome-shaped caudal appendage of lighter coloration than the rest of the body. The lateral protuberances carry external festoons. In unfed specimens, however, these protuberances are inconspicuous and there are few structural features to differentiate them from *R. capensis*. The females are even more difficult to distinguish. The lateral grooves may be more definite in *R. capensis* but this does not appear constant.

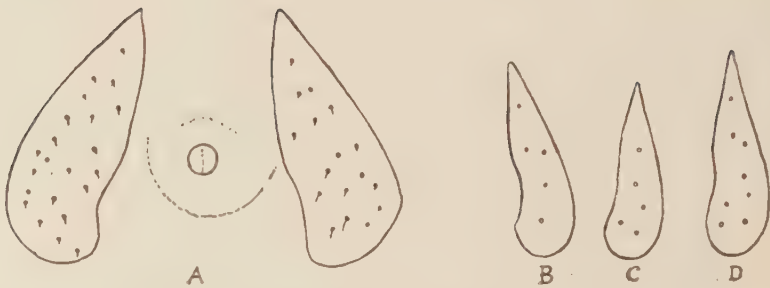


Fig. 17.—*Rhipicephalus capensis*, anal plates : A. fully engorged male and B. C. D. single anal plates from semi-engorged males.

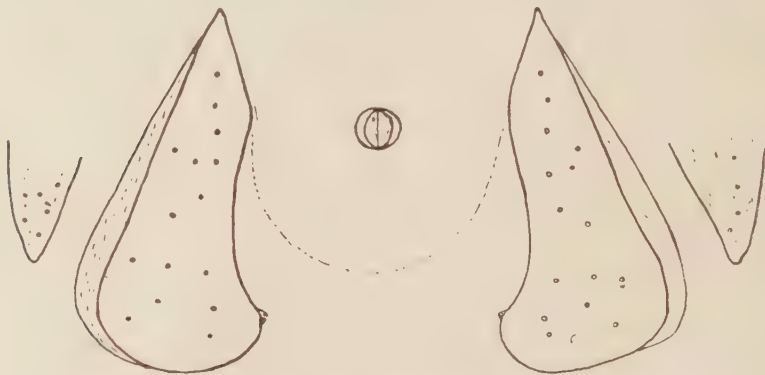


Fig. 18.—*Rhipicephalus ayrei*, anal plates of fully-engorged male.

IV—"Sanguineus" Group.

R. sanguineus Latr. does not appear to be closely related to any other *Rhipicephalus* species found in Nyasaland and is therefore placed in a separate group. It is widely distributed and has been well described and illustrated by Nuttall (1915). The main characteristics of the male scutum are the deep oval cervical pits, the unequal punctations and the three well-marked posterior dorsal forms. The scutum of the female is oval, longer than broad with long, well defined, lateral grooves and unequal punctations.

Brief biological Notes on some of the Species collected.

The present survey is an attempt to provide a check-list and host-list of the various species of IXODOIDEA present in Nyasaland and to collate the occurrence of the various species with prevailing climatic and vegetative factors. In the latter respect, these notes are merely supplementary to the data already recorded (Wilson, 1946).

The most important facts revealed were the failure of female *Rhipicephalus appendiculatus* to engorge from April to November when the relative atmospheric humidity was below 75 per cent. ; the failure of unengorged larvae to survive through the cold dry months of July ; and the limited number of hosts on which all the instars engorged (see p. 416). As a result of these limiting factors only one generation of this tick is completed under field conditions during any one year. Larvae maintained at the ordinary atmospheric temperature and humidity of the laboratory in January

to March, emerged as nymphs in from 44 to 50 days. Nymphs were common in collections throughout the dry months from April to November. During the colder months of May to August when minimum temperatures were below 50°F. they moulted to become adults in 59 to 76 days whilst during the hotter months of March to May and again in October and November this moult only required 16 days. Under optimum conditions the life-cycle from egg-laying until the appearance of the adults of the F_1 generation required 110 to 129 days but when the relative atmospheric humidity was low the cycle could not proceed as the females were unable to engorge.

Engorged female *R. neavei* were collected from September to March when the relative atmospheric humidity was low and this factor, therefore, has not such a limiting effect on the engorgement of this instar as in the case of females of *R. appendiculatus*. Under laboratory conditions, only one female oviposited and larvae emerged in from 33 to 39 days, but they failed to engorge on bovines. In Mzimba district, at an altitude of 4,500 feet, it was a common ectoparasite of the hare (*Lepus whytei*) and it was also found on cattle in Dowa District at a similar altitude, but it was absent from cattle in Lilongwe District, 3,500 feet above sea level. The site of attachment differs from that of *R. appendiculatus*, *R. neavei* being most commonly found attached to the udders and flanks of bovines and not on the ears as in the case of *R. appendiculatus*.

The most numerous collections of *R. neavei* var. *punctatus* were taken from *Lepus whytei* and from the hartebeest but it was occasionally collected in small numbers from cattle from Mzimba and North Nyasa districts. Since *R. neavei* and *R. n.* var. *punctatus* failed to breed under ordinary atmospheric conditions in the laboratory, few data on their life-history were collected.

R. simus was found on 24 occasions only amongst the hundreds of collections made during the three years of the survey; cattle were only affected on ten of these occasions and it is therefore not a common cattle tick in Nyasaland. Engorged females were found from December to May but no laboratory data could be obtained regarding its life-history. Nymphs, but not larvae, were collected from an unidentified species of field-rat.

Little is known regarding the life-history of *R. simpsoni* as it was difficult to find a host on which it would feed. In nature it appears to feed entirely on the cane-rat (*Aulacodus* sp.). Two nymphs collected in October from the cane-rat, moulted into two females in 12 and 13 days. Engorged females were collected during October and November but failed to oviposit.

Adult *R. capensis* were collected only during the period August–November when the maximum temperature was above 80°F. and the average relative atmospheric humidity during daylight hours was usually below 50 per cent. In the laboratory, females frequently oviposit but the eggs seldom hatch. The only success recorded was in October 1944 when three batches of eggs hatched as larvae, the incubation period being 35, 40 and 42 days, but in many cases emergence from the egg was incomplete and in all cases the larvae died.

R. ayrei was only collected during December and January when adults of *R. capensis* were absent, the two species therefore having a different seasonal prevalence. No engorged females suitable for breeding purposes were collected.

The life-history of *R. sanguineus* occupied 130 days, the eggs hatching as larvae in an average of 45 days, the larvae moulting as nymphs in 32 days and the nymphs moulting as adults in 37 days. Engorged females were commonly found during the hot wet months of January–March but were rare or absent during the dry months of June–December.

Amblyomma variegatum bred in the laboratory without difficulty and all instars fed on bovines. The complete life-history was as follows :—

Incubation period 83–97 days, larvae engorge three days, nymphs emerge 25 days, nymphs engorge six days, adults emerge 54–78 days (May to July), 28–35 days (August to November).

In Nyasaland, *A. variegatum* can have only one life-cycle in any one year. The females engorge and oviposit during the hot wet months of December–March. The larvae have adapted themselves to a variety of hosts and the majority engorge before August and nymphs prevail throughout most of the dry season.

Adult *Hyalomma impressum* were common on bovines during March, April and May when females were engorging. Engorged females collected during these months oviposited and larvae emerged in 59 days. Occasional specimens were collected during the remaining months of the dry season but were rare or absent during the hotter wet months of November–February. Nymphs were numerous on *Lepus whytei* during October, and adults emerged after 25–28 days. No larvae were collected during the present survey.

From field observations and from the experience gained in the breeding of the various species of tick in the laboratory, it is evident that climatic conditions in Nyasaland are best suited to *Rhipicephalus appendiculatus*, *R. sanguineus* and *Amblyomma variegatum*.

Other species, more especially *R. simus*, *R. neavei* and *R. n. var. punctatus*, exist outside their optimal conditions and serious infestations therefore never occur.

Acknowledgements.

It is a pleasure to record my most grateful thanks to Professor James Ritchie, D.Sc., for so kindly providing me with facilities at the Zoology Department, Edinburgh University, during 1945 and 1946 to enable me to complete this work. Also to Dr. A. E. Cameron, D.Sc., for his helpful criticism and advice given freely at all times.

References.

- BOARDMAN, E. T. (1944). Methods of collecting ticks for study and delineation.—*J. Parasit.*, **33**, pp. 57–59.
- COOLEY, R. A. & KOHLS, G. M. (1944). The Argasidae of North America, Central America and Cuba.—Notre Dame, Ind., Univ. Pr., 152 pp.
- MINNING, W. (1934). Beiträge zur Systematik und Morphologie der Zeckengattung *Boophilus*.—*Z. Parasitenk.*, **7**, pp. 1–43.
- NUTTALL, G. H. F. (1915). Observations on the biology of the Ixodidae. Part II.—*Parasitology*, **7**, pp. 408–456.
- SCHULZE, P. (1930). Die Zeckengattung *Hyalomma*. I.—*Z. Parasitenk.*, **3**, pp. 22–48.
- SCHULZE, P. & SCHLOTTKE, E. (1929). Bestimmungstabellen für das Zeckengenuss *Hyalomma*.—*S.B. naturf. Ges. Rostock*, (3) **2**, repr. 15 pp.
- THEILER, G. (1947). Little known Rhipicephalides.—*Onderstepoort J. vet. Sci.*, **21**, pp. 253–300.
- WARBURTON, C. (1912). Notes on the genus *Rhipicephalus*.—*Parasitology*, **5**, pp. 1–20.
- WILSON, S. G. (1946). Seasonal occurrence of Ixodidae on cattle in Northern Province, Nyasaland.—*Parasitology*, **37**, pp. 118–125.
- ZUMPT, F. (1942). Vorstudie zu einer Revision der Gattung *Rhipicephalus* Koch. VI. *Rhipicephalus appendiculatus* Neum. und verwandte Arten.—*Z. Parasitenk.*, **12**, pp. 538–551.
- ZUMPT, F. (1943). *Rhipicephalus simus* Koch und verwandte Arten.—*Z. Parasitenk.*, **13**, pp. 1–24.

THE BIOLOGY AND ECONOMIC IMPORTANCE OF *ALOMYA DEBELLATOR* (F.), A REMARKABLE PARASITE OF THE SWIFT MOTH, *HEPIALUS LUPULINUS* (L.).

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Whilst investigating the biological control of certain Hepialids, the writer reared a number of parasites from the pupae of the Swift Moth, *Hepialus lupulinus* (L.). Amongst these, one rather remarkable Ichneumonid, *Alomya debellator* (F.), attracted a good deal of notice. This particular parasite deserves attention, not only on account of its potential value in the control of Hepialid pests, but also because of certain structural peculiarities, and a very interesting life-history. Although it is not altogether unfamiliar to collectors and taxonomists, yet the exact systematic position of the species was, until fairly recently, quite undetermined. Indeed, apart from a few taxonomic notes, very little information of any kind has been published about this parasite. It is hoped, therefore, that the following account of its biology, post-embryonic development, and economic importance, may prove to be of value, not only to the scientists who will be dealing with the species in biological control operations, but also to the general reader. The paper deals mainly with the systematics, morphology, distribution, host-relationship, and life-history of the parasite. Special attention has been given to the post-embryonic stages. Finally, the economic importance of *Alomya* in relation to the biological control of the Swift Moth and certain allied Hepialid species belonging to the genus *Oncopera* in Australia, is discussed.

Systematic Position of *Alomya*.

It has already been indicated that taxonomists have found difficulty in fitting the genus *Alomya* into the general scheme of classification. Wesmael (1844) placed it in the ICHNEUMONINAE, where he gave it a tribe of its own, "*Ichneumones heterogastris*". Holmgren (Heinrich), on the other hand, thought it should go into the PIMPLINAE, while Berthoumieu (1894) assigned it to the TRYPHONINAE. According to Heinrich (1931), the genus *Alomya* has an undeniable resemblance to some rather long and slender species of the genus *Spilichneumon* Thoms.—*nonagriæ* (Holmgr.) and *johansonii* (Holmgr.)—so it is easy to understand why Wesmael and most later authors placed it in the ICHNEUMONINAE. Schmiedeknecht (1930) gives it as his opinion that, "Since it (*Alomya*) does not fit well elsewhere it should be regarded as a proper Tribe," but Heinrich (1931) leaves us with a much fuller and more satisfactory analysis of the position. "*Alomya*", says this writer, "possesses a morphological character that has hitherto escaped the notice of all authors, *on the forelegs there is only one trochanter*. This characteristic unmistakably defines the genus *Alomya*. It belongs to the same systematic group as the genera *Metopius* Panz. and *Exochus* Grav., which can be distinguished from all other Ichneumonid genera by this character. This systematic group must therefore be given, unconditionally, the rank of a sub-family, which, owing to page priority of the genus *Metopius* over *Alomya* must be called METOPIINAE. In this sub-family are included the tribes Metopiini, Exochini, Alomyini and the Australian tribe Orthognathellini. The European genera of these tribes are all parasites of Lepidoptera, the hosts of *Metopius*, being mainly Saturniids and Bombycids, whilst those of *Exochus* are Tortricids."

The exact systematic position of the genus *Alomya* may now be clearly defined as follows, Order—Hymenoptera, Family—ICHNEUMONIDAE, Sub-Family—METOPIINAE, and Tribe—Alomyini.

The genus was erected by Panzer in 1806 and the original spelling was *Alomya*. Sometimes the spelling *Alomyia* appears in the literature but this is an emendation introduced by Berthold in 1827. The tribe Alomyini is represented in the Palaearctic region by only one species, *Alomya debellator* (F.) (*ovator* auct.). *A. cruentator* Panz. according to Schmiedeknecht is difficult to classify, but certainly does not belong to the genus *Alomya*. Curtis (1826) described a new species which he called *Alomya victor* Curt., but this would appear to be only a variety of *debellator*. The species *debellator* is very variable in colour and Curtis's use of this unreliable character in defining his new species (*debellator* with a black petiole and *victor* with a red one), in view of the fact that there are already two known colour varieties of *debellator*, *nigra* Grav. and *japonica* Uchida, cannot be justified. We may therefore regard *victor* as another variety of *debellator*.

Cryptus F., *Ichneumon* L., F., Lat., Jur., have been used at various times as being synonymous with *Alomya* Panz., Fallén.

A. debellator (fig. 1) is a most striking and handsome insect. It can be recognised by the following characters: size large, up to 20 mm. in length; shape slender; wings comparatively short; antennae short and curled particularly in the female; femora and tibiae short, only one trochanter on the foreleg; body coloration mainly black, greater part of the legs and the anterior half of the abdomen reddish-brown; scutellum white in both sexes. The female can be distinguished from the male by the more curled and white-banded antennae, the larger oval shaped abdomen which is marked dorsally with several white spots on the last few segments, and by the short ovipositor.



Fig. 1.—*Alomya debellator*. Female (approx. $\times 4$).

Distribution and Host Records of the Species.

There are very few references to *A. debellator* in the literature, but all those examined indicate that this parasite is fairly widely distributed throughout western Europe. Wesmael (1844) found it on the outskirts of Brussels, and Schmiedeknecht (1903) states that the female is scarce, but the males are fairly common in dry grassy places, forest fellings, etc., which reference is taken to mean that the species also occurs in Germany, although this author does not expressly say so. In Great Britain it has been recorded from Norfolk (Curtis), and from regions so far apart as St. Kilda, an island off the west coast of Scotland, and Shere in Surrey (Waterston). My own records, obtained in the course of the present investigation, cover 21 areas in the more southerly part of England. These extended from King's Lynn in Norfolk, and Cambridge, to Dorking in Surrey. Small collections of Hepsialid larvae were also made in the Forest of Dean in Gloucestershire, and Darwen in Lancashire. From only three of these 21 areas, however, was *Alomya debellator* bred out from the collected material. The three districts were Willingham in Cambridgeshire, and Morden and Dorking, both in the county of Surrey. It is apparent from these observations that, although it has a wide range, *A. debellator* is nevertheless fairly local in its distribution. In each of the places where the parasite was found to be present, the host in every case was *Hepialus lupulinus*, and the maximum parasitism, approximately 17 per cent., occurred at Willingham.

The actual host of *A. debellator* was unknown until quite recently, and its probable identity was a matter which puzzled many collectors of the adult parasite. In 1926 Waterston wrote, "On many occasions during the last twenty years I have taken or observed this peculiar Ichneumon-fly in localities as far apart as Shere (Surrey) and St. Kilda (an island 40 miles beyond the Outer Hebrides), and in surroundings as diverse as an empty city building with its casual and temporary flora, the edge of a glade in an old-fashioned beech wood, and on an eighteen inch ledge some hundreds of feet above the Atlantic, and about one hundred feet from the cliff top. In these situations the parasite occurred in such numbers as to make me fairly certain that it was at home, and indeed the sluggishness of *Alomya* makes it unlikely that any extensive wanderings are attempted. The flight of the insect is generally slow and low, and it has a curious habit of pitching suddenly downwards and burying itself in the undergrowth. The male is more commonly seen than the female, and sometimes occurs in such numbers as to suggest that assembling is taking place. This was especially noticeable in St. Kilda where the species is common in June and July. On this island the parasites were frequently observed crawling near, or coming to the roots of docks." Wesmael came very near the mark when he said that *Alomya* was probably a parasite of a Lepidopteron, and, because of its large head, was in all likelihood cryptophagous in habit, but Stenton (in Waterston 1926) supplied the first real clue when he wrote the following note, "In 1919 I was able to establish the fact that Hepsialid larvae are the hosts of *Alomya debellator*. Males of the Ichneumon were observed assembling over the workings of a *Hepialus* sp., and in the tunnels themselves the female *Alomya* were discovered. Pairing and oviposition of the parasite were observed, and controls established to ascertain the specific identity of the host (probably *H. humuli* and *H. lupulinus*), but unfortunately both the parasitised larvae and the controls met with disaster." In the present investigation the adult parasites were actually reared from the pupae of *H. lupulinus* so that there is no longer any doubt as to the exact specific identity of the host of *Alomya debellator*.

General Biology of *A. debellator*.

The adult parasites emerge from the host pupae in June in the southern part of England. Males appear about a week before the females, the peak of emergence for the former being reached during the second week in June, and for the latter during the third week. In the laboratory, the parasites mated soon after emergence. The

males are most energetic and highly nervous in captivity, although the females have been described as somewhat sluggish and not over active in flying in their natural surroundings. Waterston's suggestion that assembling probably takes place with the males of this Ichneumonid is a most interesting one, but so far it has not been possible to confirm it.

The oviposition habits of the female were not observed but Stenton (Waterston) records that they burrow in the tunnels of *Hepialus* sp. in search of host larvae in which to deposit their eggs.

From the evidence collected during the course of the present investigation, it appears that the primary or first-instar larva of *Alomya* remains in the first stadium for a very considerable period of time. Primary larvae, obviously the product of eggs laid in the late summer and early autumn, were dissected from *lupulinus* larvae in the early spring of the year following, and even as late as the end of May. Indeed, even in the early part of June, when most of the parasites were in the mature larval or pupal stages, a few first-instar larvae were still being dissected from large host larvae which were preparing to pupate. It would appear, therefore, that the first ecdysis of *Alomya* does not take place until the host larva has reached such a stage of development that it is nearly ready to pupate. If this reasoning is correct, and the available evidence strongly supports it, then we have in *A. debellator* an example of arrested growth similar to that described by the present writer (1941) in the Braconid, *Opius ilicis*. This delay in development is of undoubted advantage to the parasite in that it ensures the maximum amount of host material for its own nourishment and subsequent growth to full size, but the probable cause and the method by which it operates is obscure. Possibly there may be a hormonal growth-inhibiting factor at work, and following on the lines of Wigglesworth (1939) on *Rhodnius*, it is possible that the presence of a large amount of moulting hormone in the host larva just prior to pupation may induce the primary larva of *Alomya* to continue its development. This, however, is a matter for further investigation.

Mature larvae of *Alomya* were found in parasitised host pupae towards the end of May, prepupae at the beginning of June, and pupae from the first week in June until the time of general emergence of the adults towards the latter part of this month. Two intervening larval stages have been dissected from the host pupae so that there are at least four, and possibly five, stadia altogether. The pupal stage lasts for a period of 11 days or longer according to the degree of temperature and the humidity. The parasite constructs a very fine translucent silken cocoon before pupating which, except at the anterior end, fits very closely to the wall of the host pupa. Parasitised host pupae can be distinguished from those that are unparasitised by their darker reddish-brown colour and by their immobility. The unparasitised pupae are light-brown in colour and are extremely sensitive, reacting violently to touch stimuli.

In captivity, the adult parasites when properly cared for will live for several months. It would seem that they are also fairly long-lived in the field judging by records of captures made in September.

Post-embryonic Development of *Alomya*.

In biological control operations, it is often necessary, and always advisable, for the research worker to be able to recognise the chief developmental stages of the

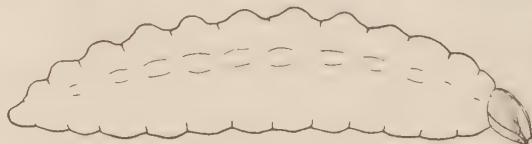


Fig. 2.—*Alomya debellator*. First-instar larva, side view. (Approx. $\times 20$.)

parasite, or parasites, which he is attempting to establish in a new environment. For this reason a fairly detailed account of the morphology and anatomy of the more important instars of *Alomya debellator* is given below.

First-instar larva.—Morphology.

The first-instar larva of *Alomya* (fig. 2) is somewhat fusiform in shape with a darker pear-shaped head and 13 greyish-white body segments. Numerous conical papillae situated in various positions on the surface of the body give this larva a very characteristic appearance. Those on the dorsal surface, which are arranged in two longitudinal rows, one on each side of the mid-dorsal line, are more prominent than the others. The largest measure 0.08 mm. in height by 0.19 mm. in diameter at the base. These larger papillae are present on segments four to 12. On the ventral surface of the body there are two rows of less prominent protuberances. Laterally, too, there are some inconspicuous raised areas, particularly on segments four to 11. The 13th or tail segment is largely composed of two posteriorly projecting lobes. A number of small spines, which are most prominent on the papillae and tail lobes, are scattered on the surface of the body.

The head is unchitinised save for the posterior margin and the cephalic skeleton. The latter (fig. 3) is well-differentiated. It consists of a pair of stout curved mandibles articulating with the supporting arms of the pleurostoma, a poorly developed and incomplete epistoma dorsally, and a thin and tenuous hypostoma ventro-laterally. In the region of the hypostoma two groups of stubby papillae are present, and on the dorsal surface of the head in the region of the epistoma a pair of fine rods or tentorial arms are produced backwards along the upper lateral margins of the head. All of these skeletal structures form a stout basket-like framework for the support of the mandibles and associated mouth parts. The unchitinised parts consist of a bi-lobed labium, each lobe with three small papillae situated near the upper margin; a pair of large uni-lobed maxillae similar in shape to the labial lobes, with four small papillae situated similarly to those on the labium; and a labro-clypeal area with a pair of

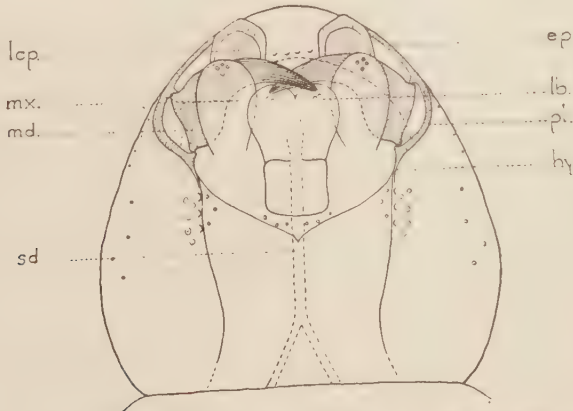


Fig. 3.—*Alomya debellator*. Head of first-instar larva, ventral view showing cephalic skeleton, etc., *lcp.* labro-clypeal projection; *mx.* maxilla; *md.* mandible; *sd.* salivary duct; *ep.* epistoma; *lb.* labium; *pl.* pleurostoma; and *hy.* hypostoma. (Approx. $\times 100$.)

curious prominent projections, which are marked with a broad chitinised margin and alternating light and dark bands within the margin. The only other features worth

mentioning are a pair of small inconspicuous dorsal papillae situated some little distance behind the epistoma, which probably represent the antennae, the salivary gland ducts uniting in the posterior part of the head to form a single duct which opens into the mouth in the region of the labium, and a number of small setae grouped on the labrum, the lateral margin of the head, and on the sub-labial area.

When fully grown the first-stage larva measures 3 mm. to 3.5 mm. in length by 0.8 mm. in maximum breadth.

With the foregoing description at hand, no difficulty should be experienced in recognising the primary larva of *A. debellator*, especially if the particular characteristics of this larva are borne in mind, namely the prominent and numerous body papillae; the bi-lobed labium and the large and prominent maxillae, which in the living larva are projected and retracted in an extremely characteristic manner; the prominent labro-clypeal projections; and the tenuous hypostoma with its bordering groups of papillae.

First-instar larva.—Anatomy.

The mouth, surrounded by the labial and maxillary lobes already described, is connected by a short fore-gut to the large and prominent mid-gut which occupies the greater part of segments two to nine and the anterior part of segment ten. This part of the alimentary tract is ellipsoidal in shape and the contents are dark-brown in colour. It is joined by a narrow neck of tissue to the short hind gut which occupies parts of segments 12 and 13. The anus is situated between the two tail lobes. In this instar the salivary glands are very large, much branched and convoluted. They lie on each side of the mid-gut in segments one to ten.

The tracheal system is well developed. It consists of two longitudinal trunks extending from segments two to 12, with extensions into the head and tail segment. There are two accessory longitudinal branches in segments two and three, a characteristic which *Alomya* shares with other Ichneumonids. Ventral and dorsal branches are given off from the main trunks in each of segments five to 12. The ventral branches unite with their fellows of the opposite side but the dorsal ones ramify into the tissues of the body. There are no spiracles in this instar.

The reproductive system is represented by a pair of club-shaped gonads, which are situated in the posterior part of segment ten, while the circulatory system consists of the usual dorsal vessel with its chambered heart and aorta.

The nervous system is composed of a series of paired ganglia, which are joined together by longitudinal connectives to form a continuous ventral nerve cord. This central nervous system is divided into three regions, the head portion consisting of the supra- and sub-oesophageal ganglia, the thoracic portion with three pairs of ganglia and the remaining abdominal region with seven pairs of ganglia in segments four to ten plus a large composite ganglion in segments ten and 11. The usual nerves are given off from these ganglia to supply the muscles and other body structures.

The mature larva.

The mature larva of *A. debellator* (fig. 4 a) is a large stoutish, greyish-white grub. It is somewhat remarkable on account of its peculiar shape, the head and first three segments being bent round almost at right angles to the rest of the body, which is rather distended posteriorly, except for the last three narrow and tapering segments. The head too is somewhat peculiar in shape in that it is flattened in front, narrow from front to back, and broad from above downwards. In an anterior view (fig. 5) it is somewhat skull-shaped, broad above and narrower below. A well developed cephalic skeleton is present. Mandibles, pleurostoma, epistoma, and hypostoma are all well marked. An unusual feature, which this stage shares with the primary larva, is the

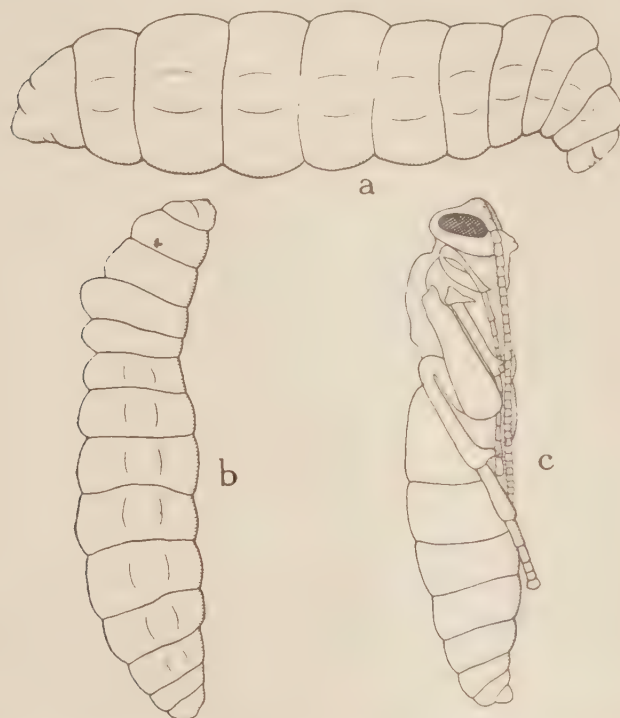


Fig. 4.—*Alomya debellator*. (a) Mature larva. Note peculiar curvature of anterior region ; (b) Prepupa ; (c) Pupa. (Approx. $\times 4$.)

complete absence of a labial sclerite or labial ring. A small ligular sclerite however is present in this instar. The labium and maxillae are well defined and both possess somewhat complex circular papillae, two on the labium and one on each maxilla. The labro-clypeal region bears two oval chitinised areas of fairly large size, one on each side of the mid-line. A pair of large, short, circular antennae are situated just above the epistoma, and just above these are two large chitinised oval patches, while in the same latitude but nearer the mid-line there are two small oval depressions. The skin of the face and of the body of the mature larva is remarkably free from spines. When feeding has finished, the larva spins a very fine silken cocoon which, except at the anterior end, fits very closely to the body wall of the host pupa.

The prepupa and pupa.

The prepupa (fig. 4 b) is very similar in many ways to the mature larva and differs mainly from it in being markedly constricted into three definite regions, head, thorax, and abdomen. The pupa (fig. 4 c) is of the ordinary Ichneumonid type. When newly formed it is white in colour except for the chocolate-coloured eyes and ocelli, but as it gets older the body colour gradually darkens and black areas appear, first on the thoracic region and later on the abdomen. The insect remains in the pupal stage for a period of 11 days, or longer, depending on the prevailing temperature and on the relative humidity.

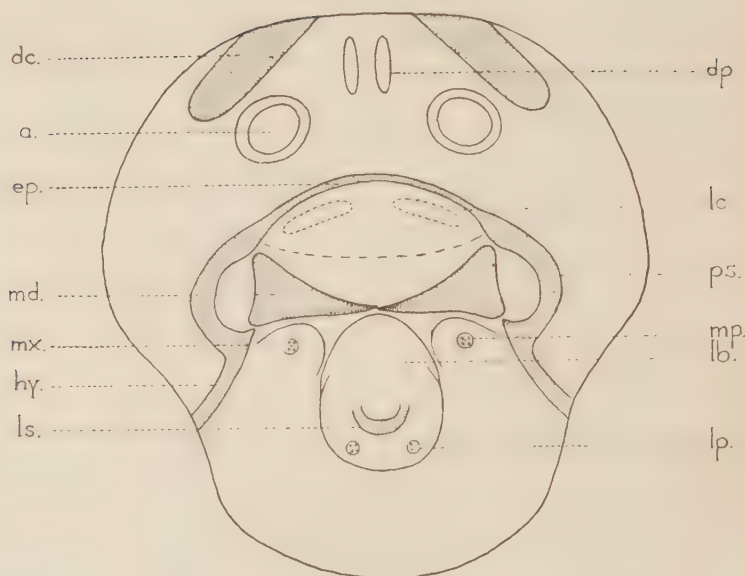


Fig. 5.—*Alomya debellator*. Head of mature larva, antero-ventral view, showing cephalic skeleton, etc., *dc.* dorsal chitinisation ; *dp.* oval depression ; *a.* antenna ; *ep.* epistoma ; *lc.* labro-clypeal chitinisation ; *md.* mandible ; *ps.* pleurostoma ; *mp.* maxillary papilla ; *mx.* maxilla ; *lb.* labium ; *hy.* hypostoma ; *ls.* ligular sclerite ; *lp.* labial papilla. (Approx. $\times 50$.)

Economic Importance of *A. debellator*.

A. debellator is the chief parasite of *Hepialus lupulinus* in the south of England, and although the number of hosts which it destroys is not large (circa 10–17 per cent.), it nevertheless plays a useful part in the control of this particular pest. In combination with other biological agencies such as entomophagous fungi, moles, insectivorous birds, Braconid and Tachinid parasites which help to reduce the numbers of *H. lupulinus*, *A. debellator* can be regarded as a valuable ally of man. It is undoubtedly the most important single factor of biological control in certain areas. It is true that its distribution is somewhat uneven, but in a properly organised scheme of control this disadvantage could be overcome by suitable introductions, and it should be remembered that the actual percentage of parasitism is not an absolute index to the true value of a parasite. Each parasite plays its own part in the scheme of control, and its own particular niche is often more important than at first glance may appear, because its total destructive effect very often cannot be altogether replaced by another agent of control.

The control of subterranean insects is generally recognised to be exceedingly difficult, and the only sound advice for anyone troubled with such pests may be, simply, to avoid those crops which are particularly favoured by the pest in question. This is one of the ways of dealing with wireworms, and the injurious species of Hepialids are in no better position. Chemical treatment has been tried, but with little success, and the difficulties and expense of the method are well known. Dressings of soot, lime, and soil insecticides have also been used, but the value of such treatment has not been demonstrated. One authority writes, "As with other soil pests, the results obtained are apt to be rather inconsistent and irregular." Tests have also been made with naphthalene, and carbon bisulphide, but here again, expense, except in small areas, where specially valuable plants are threatened, limits the use of such

chemicals. These examples will serve to illustrate the difficulties which lie in the way of effective chemical control of Hepialids. For these reasons it may be well worth while to attempt control by biological means. Indeed, a few preliminary shipments of *Alomya debellator* have already been sent from England to Australia where they will be tested against allied Hepialid species belonging to the genus *Oncopera*. Two of the main difficulties encountered in this transference of live parasites from the Northern to the Southern Hemisphere were, the non-correspondence of the seasons, and the long duration of the sea voyage. The latter difficulty caused anxiety because at no time in the life-history of *Alomya* is there a suitable quiescent stage that lasts sufficiently long to cover the time of the journey. This obstacle was overcome by selecting host pupae parasitised by *Alomya* and packing them in wood wool inside a small metal box. This box was then attached to the floor of a much larger wooden container where a quantity of raisins was available for the parasites as they emerged en route. Exit holes were bored in the small box, and the food and living space provided in the larger one were fully utilised by the adult parasites on their journey half way round the world. In this way vigorous, living adults of *Alomya* reached Australia and enabled the reactions of this parasite to its new environment, and to native species of **HEPIALIDAE**, to be tested.

A few notes on the feeding habits of Hepialids and some remarks about the area where most of the parasitised material was collected, may be inserted at this point. The larvae of the two most troublesome species of Swift Moths in this country, *Hepialus humuli* F., and *H. lupulinus*, are more or less general feeders and those of *lupulinus* in particular, attack a great variety of crops such as wheat, grass, potatoes, parsnips, carrots, lettuce, strawberries, iris, narcissus, and other bulbs, as well as peonies, dahlias, and all kinds of herbaceous plants. Certain weeds like dock, dandelion, dead-nettle, horehound, etc., are also attacked, while *humuli* is often a severe pest of the hop. In the Cambridgeshire area where most of the collections were made, *lupulinus* was found attacking *Scabiosa* and *Pyrethrum* with which large tracts of agricultural land in and around the villages of Willingham, Rampton, and Over, were planted. Hepialid material was collected in this district throughout the year, the bulk of the collecting work being carried out during the months of May and June, when the larvae of *Alomya* were more or less mature. Altogether some 7,000 larvae and pupae of *Hepialus lupulinus*, many of which were parasitised by *Alomya debellator*, were collected in this part of Cambridgeshire.

The work described in this paper was begun at the Biological Control Laboratory at Farnham Royal, and concluded in the Biology Department of St. Mary's Hospital Medical College, University of London.

Summary.

While investigating the biological control of certain Hepialids, a very interesting Ichneumonid parasite, *Alomya debellator* (F.) was reared from the pupae of the Swift Moth, *Hepialus lupulinus* (L.).

The systematic position of this parasite has been thoroughly investigated and the members of the special sub-family, **METOPINAE**, to which it has been assigned, are characterised by the possession of only one trochanter on each foreleg. *A. debellator* is the sole representative of the tribe **Alomyini**.

After extensive collecting, and a thorough search of the literature, the conclusion has been reached that the distribution of this parasite is fairly local, but its range in Great Britain and certain other European countries is wide. As a result of the present study *H. lupulinus* has been definitely proved to be the host of *A. debellator*.

A fairly full account of the life-history of the parasite has been worked out, and amongst other things, a point of special interest is the arrest in development which occurs in the first-instar larva. Possible explanations of this phenomenon are discussed.

The morphological and anatomical structure of the larva have been fully investigated and described, and useful diagnostic characteristics both for the primary and mature larval stages, have been discovered.

The paper concludes with a discussion on the potential value of *A. debellator* as a factor in the control of *H. lupulinus*, and other allied species belonging to the genus *Oncopera* in Australia. It is maintained, on the evidence collected, that this parasite in some areas is probably the most important single factor of biological control. A note on the feeding habits of Hepialids, and a few remarks about the collecting of parasitised material are appended. Altogether some 7,000 specimens of *H. lupulinus* larvae and pupae many of which were parasitised by *A. debellator* were collected from the Willingham area of Cambridgeshire.

References.

- BERTHOUMIEU, G. V. (1894). Ichneumonides d'Europe . . . —Ann. Soc. ent. Fr., **1894**, pp. 241-274, 505-592.
- BRIDGMAN, J. B. & FITCH, E. A. (1882). Introductory paper on Ichneumonidae, II.—Entomologist, **15**, pp. 84-85.
- CAMERON, E. (1941). The biology and post-embryonic development of *Opius ilicis* n. sp., a parasite of the Holly Leaf-miner, *Phytomyza ilicis*.—Parasitology, **33**, pp. 8-39.
- CURTIS, J. (1826). British Entomology, **3**, p. 120.
- HEINRICH, G. (1931). Zur Systematik der Ichneumoninae stenopneusticae, IV.—Konowia, **10**, pp. 29-30.
- SCHMIEDEKNECHT, O. (1903) Opuscula Ichneumonologica, **1**, Ichneumoninae. Fasc. 4, p. 259.
- SCHMIEDEKNECHT, O. (1930). Die Hymenopteren Nord- und Mitteleuropas, pp. 259-260.
- WATERSTON, J. (1926). A note on *Alomya debellator*, Fab.—Ent. mon. Mag., **62**, pp. 98-99.
- WESMAEL, C. (1844). Tentamen dispositionis methodicae Ichneumonum belgii.—Nouv. Mém. Acad. Sci. Belg., **18**, p. 217.
- WIGGLESWORTH, V. B. (1939). The principles of insect physiology. p. 37. London.
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AERIAL CURTAIN SPRAYING FOR LOCUST CONTROL : A THEORETICAL TREATMENT OF SOME OF THE FACTORS INVOLVED.

By K. F. SAWYER, B.Sc. *E.F.*
Ministry of Supply.

Field trials in which locust swarms were successfully attacked with insecticidal solutions sprayed from aircraft have been reported by Gunn, Lea & others (1948). In these trials the swarms were almost entirely settled on the ground. J. S. Kennedy and B. A. Toms, in an unpublished report, suggested, however, that aircraft spraying could be readily adapted for attacking fully flying swarms. Such an adaptation, the *aerial curtain* method, has not yet been properly tested, but it has been carefully examined by Ward (Gunn, Graham & others, 1948) in the light of all the field experience available. Ward has given a method of calculating approximately a number of the quantities involved, such as the weight of spray mixture required per unit length of curtain and the interval that must elapse between successive spraying runs. A different and essentially dynamic method of calculating these quantities is presented in the following pages. It leads to a clearer picture of the events occurring within the curtain and forms a more precise means of calculating the quantities involved than Ward's conception of the steady mean of locust dose.

General Principles.

For simplicity of treatment an ideal swarm is postulated, *i.e.*, one with a fairly well-defined length, breadth, and depth, moving at a constant speed and preserving the same general direction of flight for a reasonable length of time. Under such conditions, the attack begins with an aircraft flying above, across and ahead of the advancing front of the swarm and emitting an *element* of spray curtain equal in length to the projected breadth of the swarm. This element will consist of spray droplets of a wide range of sizes so that, falling with a corresponding range of terminal velocities, the droplets will soon be distributed through a vertical distance comparable with the depth of the swarm. This first element will be reinforced, at predetermined *renewal intervals*, by further elements emitted along the same line in the air mass during succeeding aircraft runs, so as to build up and maintain a *curtain* of specifiable density or performance. As the swarm passes through the curtain, each locust will pick up, by sedimentation or collision, a certain number of spray droplets and, if conditions within the curtain have been correctly adjusted, this number of droplets will be sufficient to impart to the locusts a lethal dose of the insecticide dissolved in the liquid.

The concept of an attack along these lines is essentially a simple one, but the practical success of an actual operation depends upon the product of a large number of factors (see Gunn, Graham & others, 1948). Amongst these factors, the effects of which should be statistically predictable, are the size distribution of the spray droplets and the quantity of spray deposited on a flying locust under a given set of conditions. Combining these with some necessarily assumed geometrical regularity in the swarm, it is possible to investigate theoretically the performance to be expected in an ideal case, and to indicate from this the directions in which adjustments might have to be made to meet conditions in the field. The following analysis is made in greater detail than hitherto and the results presented graphically in a form suitable for use in the field.

Method of Calculation.

As a preliminary, idealise the conditions and consider a rectangular prism of one metre square section, extending from the ground to the spraying height of the aircraft, with two of its faces parallel to the aircraft course. As the aircraft passes over the topmost cubic metre of this prism, a one metre length of spray emission, W grams, will enter the prism, where W is calculated from the known speed of the aircraft and the rate of emission of liquid. The average volume concentration of the spray at the top of the prism is thus initially $W \text{ g/m}^3$. In still air, the average concentration at any lower point in the prism, as the droplets fall, will be solely a function of the size of droplets into which the liquid is disrupted and the time which has elapsed since the passage of the aircraft. In practice the air will be in turbulent motion; local variations in concentration will be produced and some of the droplets will be carried out of the prism in all directions. If the prism is not very close to the ends of the curtain element, the loss in directions parallel to the length of the element will, on the average, be balanced by counter-diffusion from neighbouring prisms. Diffusion at right angles to the length of the element will increase the thickness of the element. Since this effect decreases the concentration per unit volume, but correspondingly increases the time it takes the insect to fly through the element, the product of concentration and time will remain constant and diffusion in this direction, too, can be neglected. It is therefore possible, without undue loss of generality, to replace the practical case in this analysis by an idealised one in which each metre length of spray is confined within its metre square prism, which is moved forward with the speed of the wind. The actual value of the wind speed is unimportant since it acts equally upon the locust and the prism. It is only necessary to take into account the relative motion of the two, *i.e.*, the true horizontal airspeed of the locust.

Let δW be that part of W which is broken into droplets whose diameters lie in the narrow range between d_1 and d_2 cm. Assuming no loss by evaporation, droplets of diameter d_1 and d_2 will fall with terminal velocities V_1 and V_2 m./sec., respectively, and they will acquire these steady values after falling only a few metres. At the end of any interval T seconds after W was emitted, the droplets of diameters d_1 and d_2 will be separated by a vertical distance $T(V_1 - V_2)$ metres and the average volume concentration, C , in this region of the prism will be—

$$C = \frac{\delta W}{T(V_1 - V_2)} \text{ (g/m}^3\text{)}$$

or, writing δV for $V_1 - V_2$

$$C = \frac{1}{T} \cdot \frac{\delta W}{\delta V} \text{ (g/m}^3\text{)} \dots \dots \dots (1)$$

Let d be the mass median diameter of the droplets in the elementary range d_1 to d_2 and let V be the terminal velocity of this droplet size. Then in time T , droplets of diameter d will have fallen a distance D below the release point given by—

$$D = VT \text{ (metres)} \dots \dots \dots (2)$$

Eliminating T between (1) and (2)—

$$C = \frac{V}{D} \cdot \frac{\delta W}{\delta V} \text{ (g/m}^3\text{)} \dots \dots \dots (3)$$

If δW is taken sufficiently small, all the droplets giving rise to the value of C in (3) will be falling at velocities not very different from V , so that approximately all the droplets in V cubic metres of the prism will fall on one square metre of horizontal surface in one second at the distance D below the release point. The mass rate of sedimentation, S , at D is therefore :—

$$S = VC = \frac{V^2}{D} \cdot \frac{\delta W}{\delta V} \text{ (g/m}^2\text{/sec.)} \dots \dots \dots (4)$$

If a locust flies through the prism at D , it will pick up, by sedimentation and collision, a certain quantity Q_0 of the spray liquid. The relationship of Q_0 to S , for a given set of conditions, is too difficult to derive analytically, but can be determined experimentally (Kennedy, Ainsworth & Toms, 1948). It takes the form—

$$Q_0 = S.A(d, u).t \text{ (grams)}$$

where $A(d, u)$ is called the *equivalent area* of the locust and t is the time taken by a locust to traverse a metre cube, i.e., $t = \frac{1}{u}$ where u m/sec. is the true airspeed of the locust. The equivalent area, as experimentally determined, is the horizontal plane area which, when passed through a curtain of droplets of diameter d with the same velocity as the locust, collects the same total number of droplets as the locust. The equivalent area is clearly a function of d and u , since it is not necessarily the plan area of the locust, but takes into account all the droplets collected by collision due to the airspeed of the locust as well as by sedimentation due to the fall of the droplets.

Inserting the equivalent area into equation (4), the quantity of spray picked up by a locust flying normally through the prism at D becomes—

$$Q_0 = S.A(d, u).t = \frac{V^2}{Du} \cdot \frac{\delta W}{\delta V} \cdot A(d, u) \text{ (grams)} \dots \dots \dots (5)$$

The value given by (5) refers, of course, to a single locust flying through the prism at D , but in practice a swarm will not be worth attacking unless it has an appreciable density—i.e., there is an appreciable number of locusts per unit volume. The quantity of spray picked up by the locust's passage at D will then be less than that given by (5) by the amount picked up by all the locusts in the prism between D and the top of the swarm. The *attenuation* of the spray produced in this way can be calculated approximately by assuming that the locusts are randomly distributed within the swarm and that there is always sufficient turbulence in the air to mix the droplets thoroughly after the passage of each locust—i.e., the locust leaves no appreciable droplet shadow. Since a displacement of only a few centimetres is involved in fulfilling this condition, it should be reasonably satisfied by all the droplet sizes here considered. On this basis, the attenuation follows a logarithmic law and if the top of the swarm is at a vertical distance D_0 below the release point, the quantity of spray reaching the point D in a swarm will be decreased by the *transmission factor*—

$$\alpha = -\exp \sigma(D-D_0)$$

where σ is the total intercepting area of the locusts in unit depth of the prism. If there are, on the average, n locusts per cubic metre of swarm, nu locusts will pass through each cubic metre of the prism per second and since each locust presents an equivalent area of $A(d, u)$ for $1/u$ seconds, the total intercepting area per metre depth of prism is—

$$\alpha = nu \cdot \frac{1}{u} \cdot A(d, u) = nA(d, u)$$

and the transmission factor, α , at a depth $(D-D_0)$ in the prism becomes—

$$\alpha = -\exp[n(D-D_0).A(d, u)] \dots \dots \dots (6)$$

Combining (5) and (6), the quantity of spray, Q , picked up at D by one locust as part of a swarm flying normally through the prism is given by—

$$Q = \alpha Q_0 = \frac{V^2}{Du} \cdot \frac{\delta W}{\delta V} \cdot A(d, u) \left\{ -\exp[n(D-D_0).A(d, u)] \right\} \text{ (grams)} \dots \dots \dots (7)$$

The quantity $n(D-D_0)$ above is identical with N the total number of locusts per square metre plan area of the swarm and is very roughly equal to the apparent

density when the swarm is viewed in plan (*cf.* the *area density* of Gunn, Perry & others, 1948).

By the argument advanced earlier, the concept of a prism can now be dispensed with and Q , given by (7), represents the quantity of spray deposited on a locust flying at right angles to the line of emission through one curtain element. An oblique path through the element increases the dose proportionately. The small section at each end of the curtain element, where the concentration is materially reduced by lengthwise diffusion, is here neglected.

It is implicit in (7) that turbulence, though sufficient to cause some diffusion of the droplets, is not so great that they are appreciably retarded or accelerated in their fall. In terrain where convection currents are pronounced, the expression is invalid. Within this limit of turbulence, equation (7) shows that a locust, as it passes through each curtain element, will be dosed only with droplets of a narrow size range.

The form of equation (7) shows that the dosage imparted by the element to the swarm will vary over a fairly wide range both in space and in time. As the element sinks through the swarm, the dosage at D will increase from zero to a maximum value and then decrease again in a time, and in a manner, determined by the spray droplet size distribution. As the dosage decreases, there will come a time when the value of Q at a given depth reaches the minimum permissible for a given lethal effect and it will then be necessary to supplement the first element by one or more succeeding elements if the required performance is to be maintained. This requires careful adjustment of the weight of material in the elements and the renewal interval between them. To do this to the best advantage, a complete solution of equation (7) is required giving the spatial and temporal distribution of Q for a swarm of given characteristics. Such a solution can readily be derived graphically and, if a suitable plotting grid is employed, a very clear presentation of the results can be obtained.

Details of the Graphical Solution.

In order to illustrate the method of solution and to provide a basis for the discussion of other factors at a later stage, two cases will be worked out in detail, each based on

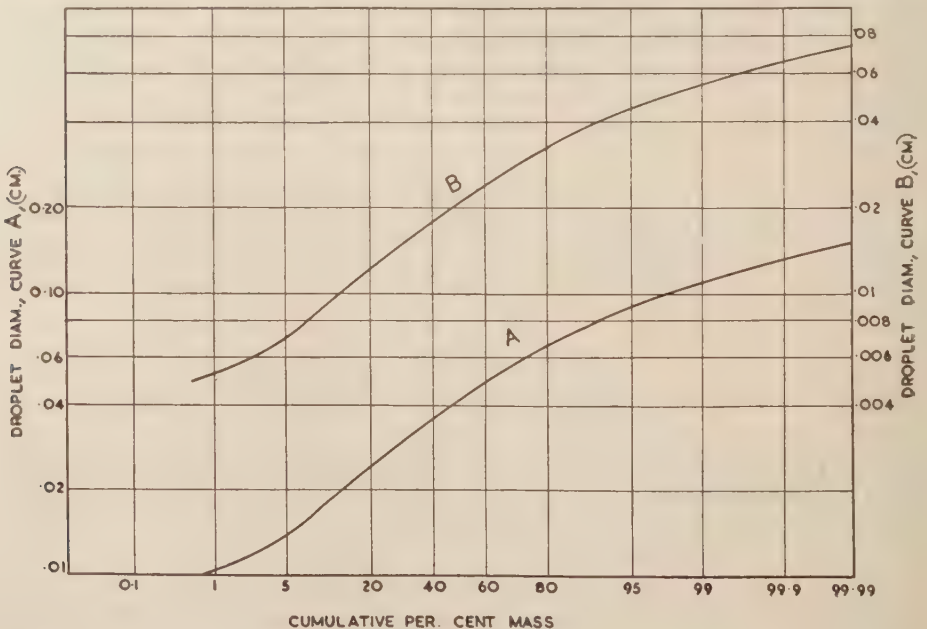


Fig. 1.—Droplet size distribution of coarse (A) and fine (B) sprays plotted on a logarithmic probability grid.

a particular spray droplet size distribution. These distributions will be called, purely for the purposes of distinction, *coarse* and *fine*. The droplet size distribution of the coarse spray is given as curve A in fig. 1, where cumulative per cent. mass is plotted against droplet size on a logarithmic probability grid. Apart from a little smoothing of the curve, this is substantially the size distribution of the spray which proved so successful in the recent air-to-ground spraying trials against the Red Locust, *Nomadacris septemfasciata* (Serv.), in the Rukwa Valley, Tanganyika (Gunn, Lea & others, 1948). The size distribution of the fine spray, curve B in fig. 1, is derived arbitrarily from that of the coarse spray by halving the droplet sizes throughout. It probably represents the lower limit of fineness in anti-locust spray obtainable by the present method and equipment.

The spray liquid is here considered to be involatile. In practice, there may be a small loss by evaporation; but as this will not usually affect the poison content of the droplets nor alter appreciably their terminal velocities, the effects of evaporation can be neglected without appreciable loss in generality.

Terminal velocities for a range of droplet sizes are given in Table I and are based on the data of Fuchs (1936) and of C. N. Davies (unpublished report, Ministry of Supply).

TABLE I.

Droplet diam.	Terminal Velocity
0.15 cm.	5.45 m/sec.
0.10 "	4.00 "
0.075 "	3.13 "
0.05 "	2.10 "
0.025 "	0.94 "
0.010 "	0.27 "

Small corrections for density: $T.V. \propto \sqrt{\rho}$

Very few measurements of the equivalent area of the locust have been reported. Those used in the present instance were separately determined by a method similar to that described by Kennedy, Ainsworth and Toms (1948). These measurements

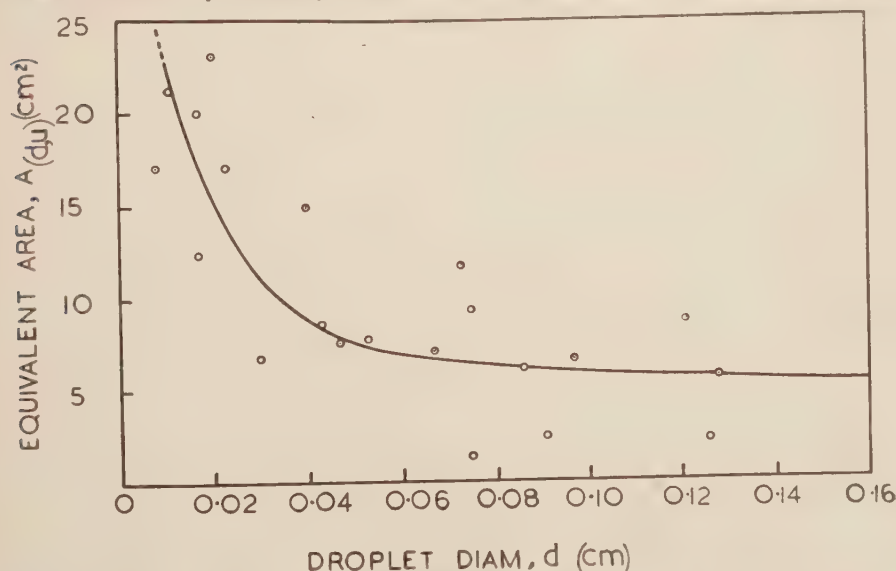


Fig. 2.—Equivalent area of *Locusta migratoria migratorioides*, as function of spray droplet size, determined experimentally for a locust airspeed of 3 m/sec. (6.7 m.p.h.).

were made with *Locusta migratoria migratorioides* (R. & F.), flying at an airspeed of 3 m./sec., and the values obtained are given in fig. 2. For the finer droplets, the values in fig. 2 are much smaller than those reported by Kennedy, Ainsworth and Toms (1948), but the agreement improves towards the larger sizes and is good for droplets greater than 0.05 cm. diameter. This disagreement is not unexpected in view of the practical difficulties of the experiment and the variability of the material. In the circumstances, fig. 2 should be regarded only as illustrative of the way in which equivalent area is related to droplet size: it is unlikely to be quite the same for other species of locust or other insecticide solutions.

The first step in the detailed calculation is the completion of a table, part of which is reproduced as an example in Table II. For the first column W , the weight of spray per metre, has been divided into 20 equal parts corresponding to the δW 's of equation (7). These intervals are sufficiently frequent for most practical purposes, except perhaps for the few very large drops. From fig. 1, curve A, column 2 is completed by entering the bounding sizes of each 5 per cent. of the cumulative mass. From Table I the terminal velocities corresponding to these droplet sizes are entered in col. 3, and their differences when taken in pairs (to give the δV values) in col. 4. Next, the droplet sizes corresponding to $2\frac{1}{2}, 7\frac{1}{2}, \dots$ per cent. cumulative mass (representing d) are entered in col. 5 and the terminal velocities of these droplets (V) in col. 6. The

TABLE II.
Size distribution: fig. 1, curve A. $W=300$ g/m. $\delta W=15$ g/m.

1	2	3	4	5	6	7	8	9	10
δW as % of W	Bounding sizes of δW d_1 d_2	Terminal Velocities v_1 v_2	δV	d	V	$V_2 \frac{\delta W}{\delta V}$	$\frac{T}{(D=100)}$	$\frac{1}{u} A(d, u)$	$\frac{V_2 \delta W}{u \delta V} A(d, u)$
100 to 95	0.15 0.09	5.45 3.65	1.8	0.10	4.00	133	25	2.0×10^{-4}	26.6×10^{-3}
95 to 90	0.09 0.08	3.65 3.27	0.38	0.083	3.40	458	29	2.1 "	95.9 "
90 to 85	0.08 0.072	3.27 2.96	0.31	0.075	3.08	459	32	2.1 "	96.3 "
10 to 5	0.018 0.014	0.60 0.40	0.20	0.016	0.50	19	200	5.9 "	10.2 "
5 to 0	0.014 0.005	0.40 0.07	0.33	0.013	0.35	6	286	6.9 "	4.2 "

values of $V \frac{\delta W}{\delta V}$ for col. 7 are obtained from cols. 1, 4 and 6, and finally col. 8, giving the time each droplet size takes to fall a convenient distance (say, 100 metres), is calculated from col. 3. The values of $A(d, u)/u$ in col. 9 are inserted from fig. 2 and col. 10 is obtained from the products of cols. 7 and 9.

From equation (5), the values in col. 10 correspond to the product $D \times Q_0$ so that the first group of figures gives the value of D at which Q_0 falls to 10^{-3} g. Fig. 3 displays the information more clearly.

The ordinate in fig. 3 represents D , measured from the top left-hand corner where the release point is situated. The abscissa represents the time, T seconds, after spray emission. The locus of a falling droplet, say one of 0.1 cm. diameter, is represented by a ray issuing from the release point and intersecting the horizontal line through $D=100$ at $T=25$, since a droplet of this size falls 100 m. in 25 seconds. Rays corresponding to all the other droplet sizes in Table II are drawn in on a similar basis from col. 8.* As each group of droplets falls (*i.e.*, symbolically, along each ray)

*For clarity in fig. 3 and following diagrams many of the rays are indicated only by stub lines at the edges.

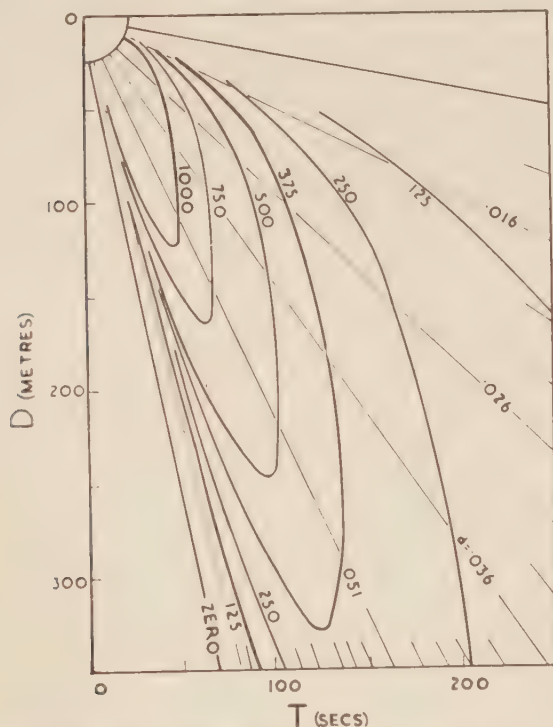


Fig. 3.—Dose deposited on a single locust flying through one curtain element of the coarse spray as a function of D , T and droplet size (cm.). Contour values are in μg . (10^{-6}g).

Q_0 varies inversely with D and in particular reaches 10^{-3} g . at the values of D given by the first group of numbers in col. 10. These values of D (measured parallel to the D axis) are marked off on the appropriate rays and joined by a smooth contour line (justified by the continuous nature of the droplet size spectrum). This is the line marked $1,000\ \mu\text{g}$. in fig. 3. At all points in the diagram inside this line Q^0 exceeds $1,000\ \mu\text{g}$.; outside it Q^0 is always less than this value. Using the inverse variation with D and the $1,000\ \mu\text{g}$. line, contours for other values of Q^0 are drawn in.

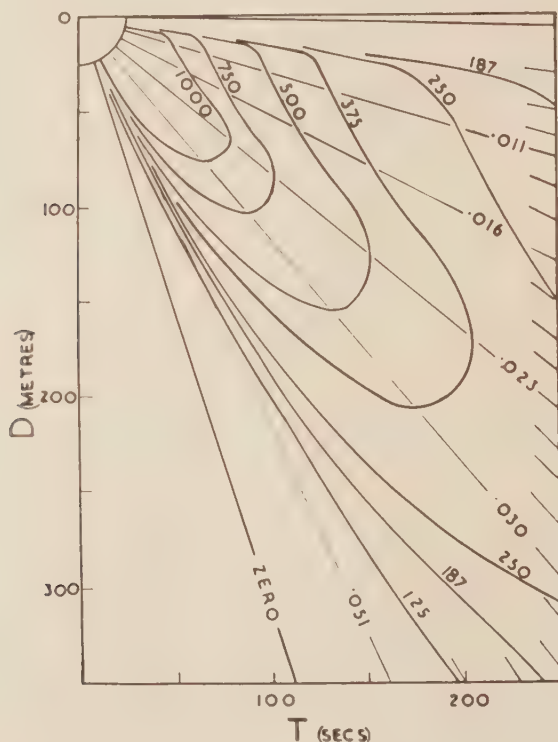
In fig. 3, the curtain element itself is represented at any instant by a thin vertical line bounded at the top and bottom by the zero contour lines and, starting from the release point, moving steadily across the diagram in accordance with the time scale below. The intersection of this line with the contours gives the instantaneous values of Q_0 as a function of D .

A corresponding diagram is given in fig. 4 for the fine spray. Since Q_0 is the dosage deposited on a solitary locust flying through the element, figs. 3 and 4 represent the *potential* dosing power of the element, characteristic of the associated spray droplet size distribution. Note the impression of much greater penetrating power associated with the coarse spray in fig. 3.

The distribution in space and time of the dosage, Q , imparted by an element to a swarm is calculated in a similar way. Taking a pair of fixed values for n , the number of locusts per cubic metre and \bar{D}_0 , the spraying height, the transmission factor, z , is calculated from equation (6) for various depths within the swarm, usually at

intervals of 25 or 50 m. Then from col. 10 of Table II, a plot of αQ_0 against D for each ray gives the rate at which Q diminishes along that ray and the points at which Q falls to selected values. These are marked off and points of equal dosage on each ray joined by a smooth contour line. The resulting diagram is a graphical solution of equation (7).

In figs. 5-14, diagrams constructed in the above manner are given for $n=5$, 15 and 45 and $D_0=50$, 100 and 200 metres. Comparing these diagrams with figs. 3 and 4, note how greatly the contours have been altered in size and shape by taking into account the effects of attenuation. Evidently attenuation is a major factor in the performance of a curtain element.



Taking fig. 5 again, the 125 $\mu\text{g.}$ contour has reached a depth of 90 m. in the swarm at 50 seconds. Now in this 50th second, a 3-metre length of swarm ($u=3$ m/sec.) will pass through the curtain element so that all the locusts in a volume of $3 \cdot 90$ cubic metres of swarm will receive a dose in excess of 125 $\mu\text{g.}$ from a metre length of element. At any instant, the intercept on the ordinate between the top of the swarm and a contour line is thus proportional to the number of locusts receiving in one second a dose in excess of the contour value. Each locust becomes, in effect, a fixed point in the diagram corresponding to the time and depth at which it passed through the element. It follows that the swarm itself is fixed relative to the diagram, so that the

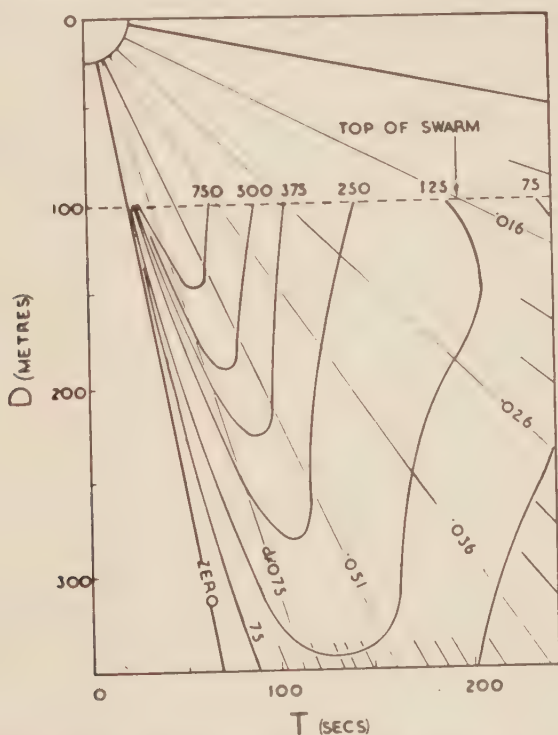


Fig. 5.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0=100$ m. and $u=5$ locusts/ m^3 . Contour values are in $\mu\text{g.}$

area enclosed by a given contour and the top of the swarm is directly proportional to the total number of locusts dosed to at least the contour value. Moreover, the shape of the contour line is the *shape* cut out by that dosage within the swarm, and a scale can conveniently be given to these shapes by multiplying the T scale by u and reading metres instead of seconds. These are valuable properties of the method of plotting used here and lead to an almost pictorial representation of the course of events within the curtain element. The method also allows the summed effects of a number of elements emitted in succession to be obtained with equal clarity.

Combination of Elements into Curtains.

The performance of a curtain built up from a regular sequence of elements will depend, other things being equal, upon the renewal interval between the elements. Too short an interval will result in wasteful overdosing, too long an interval in patchy

mortality. Ward in his analysis (Gunn, Graham & others, 1948) has taken the *steady mean* of locust dose as his criterion of satisfactory performance and determines his renewal interval accordingly. This leads to the requirement that some five or six elements shall be emitted in normal sequence well ahead of the swarm so that, by the time the swarm enters it, the curtain has already been built up and the dose deposited on the locusts fluctuates only within a limited range about the steady mean value. This steady mean spreads rather slowly through the large bulk of the curtain and to maintain the dose fluctuations within reasonable bounds, the tendency is always towards the use of small rates of spray emission and short renewal intervals. A necessarily excessive dosage is applied at the top of the swarm so that it shall fall off steadily within the swarm until it reaches the minimum lethal value at the bottom. Ideally, the contours of equal dosage should be horizontal lines in the swarm.

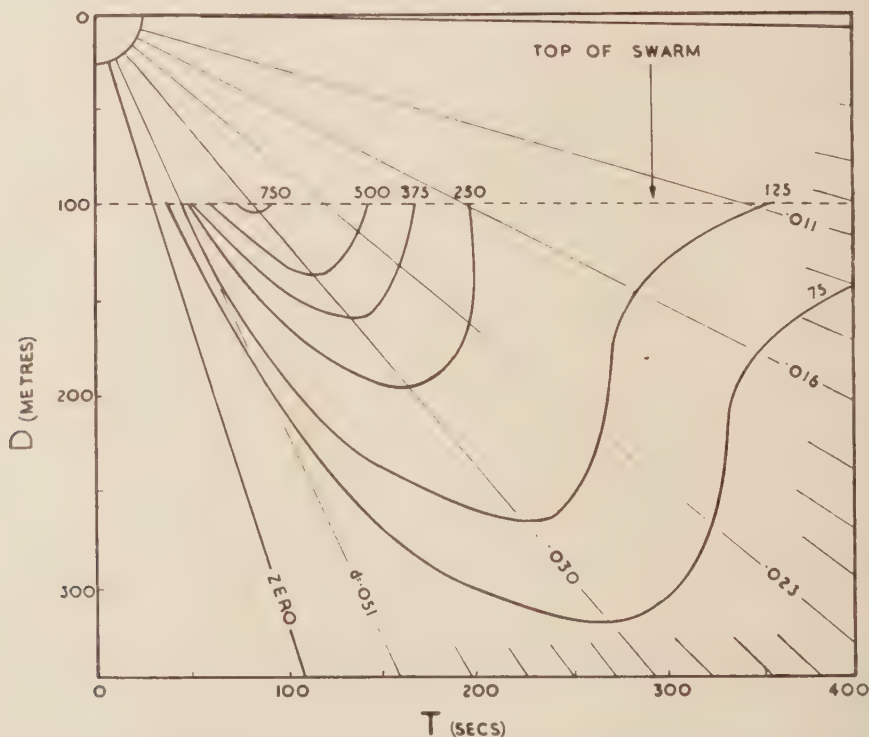


Fig. 6.—Dose deposited on locusts in a swarm flying through one curtain element of the fine spray when $D_0=100$ m. and $n=5$ locusts/m³. Contour values are in μg .

The present treatment is a more detailed one and brings out a number of points not apparent or inadequately allowed for by the steady mean method. Figs. 5-14 show that the zones of equal dosage produced by each element are roughly U-shaped, with a tendency for the dosage to fall off as much along the length of the swarm as through its depth. Evidently, big fluctuations in dosage at the top of the swarm can be permitted without producing undesirably large variations lower down and the even grading of dosage from top to bottom of the swarm is not essential for satisfactory performance.

With the insecticide used recently in East Africa (Gunn, Lea & others, 1948), it has been shown that some 100 to 200 μg . of spray deposited on each locust produce mortalities of over 90 per cent. Taking this as a rough basis for discussion, it is clear from figs. 5-14 that a single curtain element is of itself capable of imparting a lethal dose to a substantial number of locusts. Moreover, this dosage level reaches well down into the swarm within a fairly short time after spray emission and is

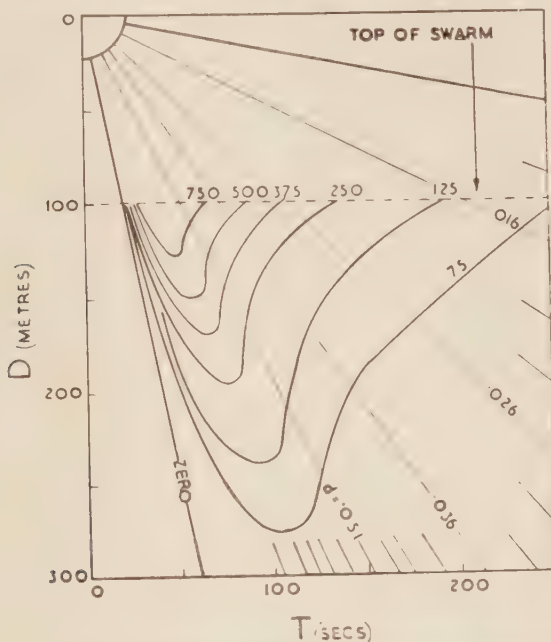


Fig. 7.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0 = 100$ m. and $n = 15$ locusts/ m^3 . Contours in μg .

maintained for a useful period of some 50 to 150 seconds. Each element can, as it were, stand by itself. There is no necessity to build up the curtain ahead of the swarm and the elements can be emitted at much longer intervals than the *steady mean* method would suggest. This makes it practicable, in favourable conditions, to maintain a curtain of limited length with only a single aircraft.

Figs. 5-14 clearly suggest that the curtain should be built up on the basis of dosage zones. The renewal interval is then determined by the extent of the overlap of succeeding elements required to ensure that no part of the swarm receives less than the desired dose. This overlap can readily be determined by taking a few replicas of the appropriate dosage diagram and arranging them side by side at such a spacing that the sum of the overlapping contours reaches the required value at a given depth. The spacing between the diagrams, measured on the T axis, gives the renewal interval. It will not always be possible in overlapping to take full advantage of the slowly decreasing *tails* of preceding elements because the dosage builds up again very quickly with the arrival of each new element. In these cases, the dosage is almost completely derived from the overlap of two elements only, so that the renewal interval can be read directly from the one diagram. If the required contour does not reach to the bottom of the swarm, the weight of the element may be increased. The contour values and the weight of spray emitted are, of course, directly proportional.

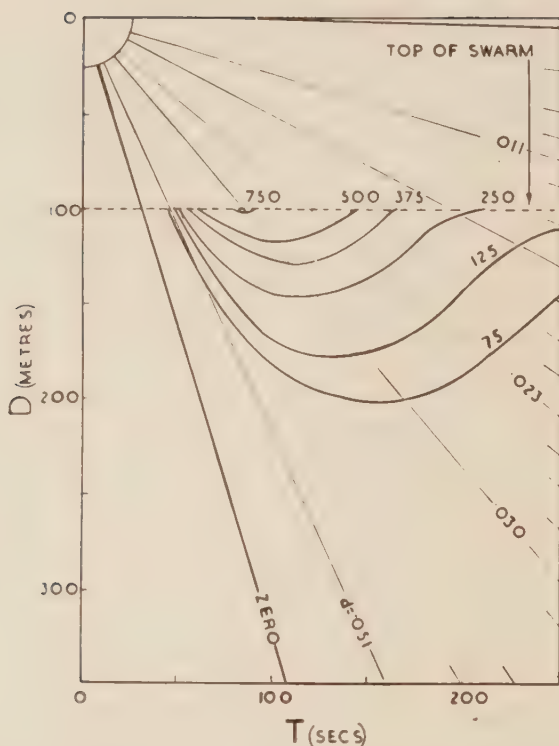


Fig. 8.—Dose deposited on locusts in a swarm flying through one curtain element of the fine spray when $D_0=100$ m. and $n=15$ locusts/m³. Contours in μg .

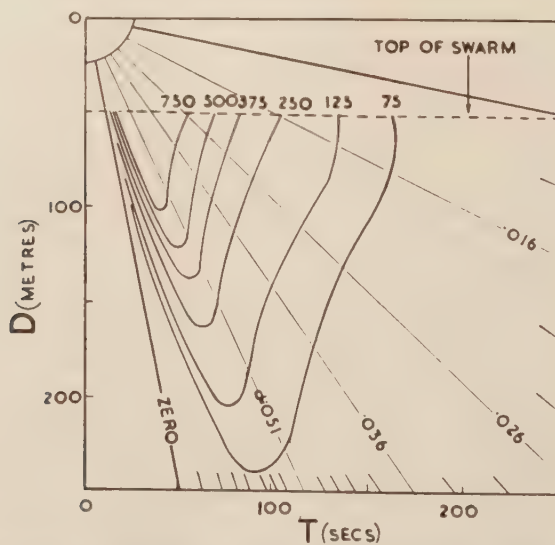


Fig. 9.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0=50$ m. and $n=15$ locusts/m³. Contours in μg .

The above procedure is very suitable for field use. With the aid of diagrams similar to those of figs. 5-14, but calculated for the particular droplet size spectrum to be used and the species to be controlled, it should be a relatively simple matter to make a last minute adjustment to quantities in the field and so adapt the curtain to the individual characteristics of the swarms as they are encountered. Some flexibility of this nature is essential to successful operation.

Performance as affected by Droplet Size Distribution and other Factors.

Qualitative differences in performance between the two droplet size distributions will already have been noticed in the diagrams discussed above, but it is difficult to formulate a quantitative expression of them. From general considerations, however,

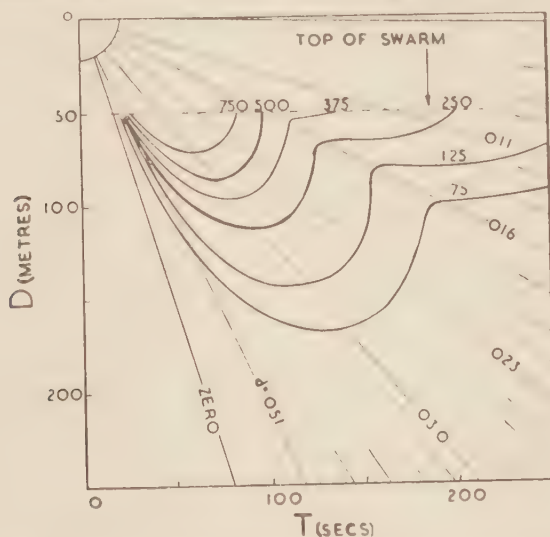


Fig. 10.—Dose deposited on locusts in a swarm flying through one curtain element of the fine spray when $D_0 = 50$ m. and $n = 15$ locusts/m³. Contours in μg .

it seems most probable that the choice of a size distribution would turn largely upon the length of the renewal interval it permitted and the efficiency of the operation in terms of the number of locusts killed, under comparable swarm conditions, with a given weight of poison.

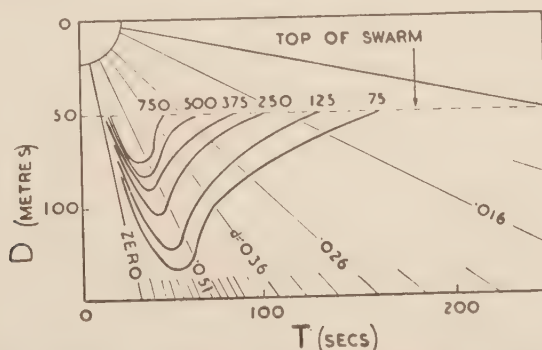


Fig. 11.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0 = 50$ m. and $n = 45$ locusts/m³. Contours in μg .

Taking the renewal interval criterion first, fig. 15 shows how this interval, R , is related to the depth of the swarm ($D-D_0$), and the number of locusts per square metre plan area (N), the full lines of the figures corresponding to the coarse size distribution and the broken lines to the fine. The renewal intervals have been calculated on the basis that the $125 \mu\text{g}$. contours must just coincide at the bottom of the swarm. This is a simplified version of the procedure given earlier, but is sufficient for the purpose. It corresponds, allowing for overlap, to a minimum dosage of some $150 \mu\text{g}$. of spray for an output (W) of 300 g/m .

If reference is made to fig. 15 for quantitative differences, its general indications are as follows:—

1. For a constant value of W and a spraying height not greater than 100 m ., the fine distribution permits renewal intervals (figs. 15 A and B) about twice those

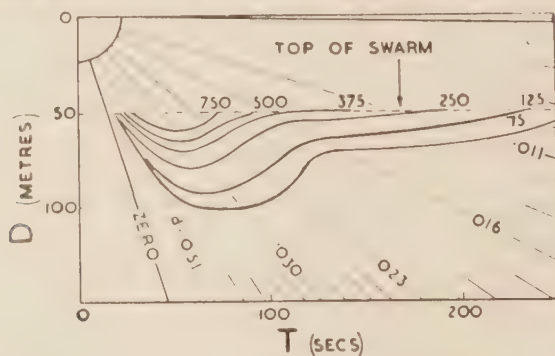


Fig. 12.—Dose deposited on locusts in a swarm flying through one curtain element of the fine spray when $D_0=50 \text{ m}$. and $n=45 \text{ locusts/m}^3$. Contours in μg .

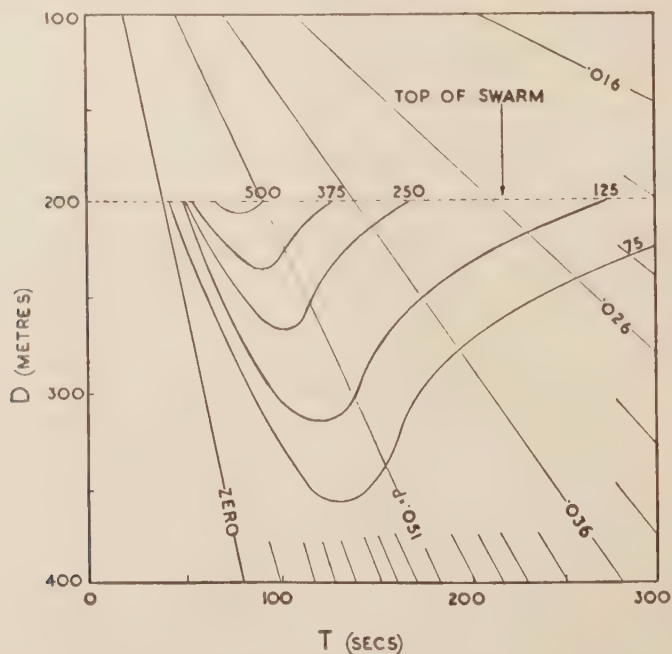


Fig. 13.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0=200 \text{ m}$. and $n=15 \text{ locusts/m}^3$. Contours in μg .

required for the coarse distribution for very shallow swarms. For intermediate depths of swarm, the renewal intervals of the two distributions do not differ significantly. Only the coarse distribution can deal with deep swarms.

2. The depth of swarm which can be effectively dosed with a constant W decreases rapidly with increase of locust density.

3. Reduction of the spraying height to 50 m. improves the performance of the fine distribution, but has the opposite effect on that of the coarse distribution.

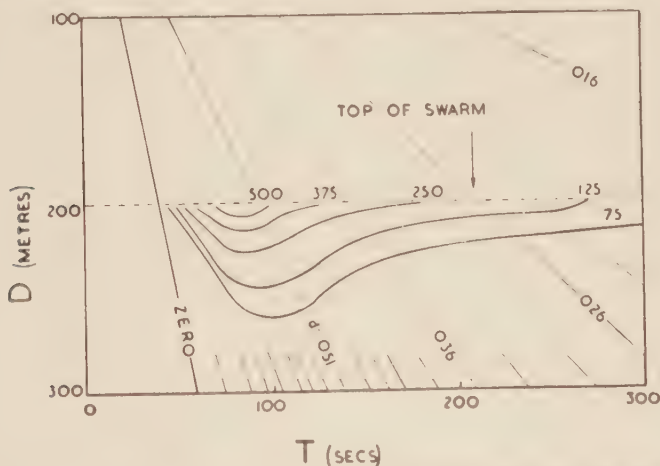


Fig. 14.—Dose deposited on locusts in a swarm flying through one curtain element of the coarse spray when $D_0=200$ m. and $n=45$ locusts/m². Contours in μg .

4. Increasing the spraying height to 200 m. increases the renewal interval permitted for the coarse distribution with shallow swarms, but reduces penetration into deep swarms. The renewal intervals are then nearly identical with those of the fine distribution sprayed from 50 m. There is an optimum spraying height for each size distribution.

5. Except for both extremes of depth, the differences in the renewal intervals of the two distributions for any given swarm are such that they could be brought into coincidence by an appropriate adjustment of W , and there is accordingly no outstanding point in favour of either distribution. For extremes of depth, the drop size distribution would be important.

The other criterion, the efficiency of the spraying operation, is illustrated in fig. 16. The number of locusts dosed to at least $125 \mu\text{g}$. by one metre length of element, $N_{Q>125}$ is given by the product $3n R(D-D_0)$, since $3n$ locusts fly through each metre of the element per second ($u=3$ m/sec.) and the element maintains a dosage in excess of $125 \mu\text{g}$. for R seconds at the bottom of a swarm $(D-D_0)$ metres deep. Assuming a dose of $125 \mu\text{g}$. to be fatal, $N_{Q>125}$ is the number of locusts killed by 300 g. of spray. If applied uniformly, this 300 g. would provide 2.4 million lethal doses. The ratio of $N_{Q>125}$ to 2.4 million is thus a measure of efficiency.

In fig. 16, the efficiencies of the curtains considered in fig. 15 are plotted against $D-D_0$ and N . In each case the efficiency rises steadily to a maximum of about 10 per cent. corresponding to an optimum combination of spraying height, swarm depth and density. Just beyond this optimum condition the efficiency falls rapidly because the swarm is then too deep for that particular spray size distribution and to reach the few locusts at the bottom the remainder are grossly overdosed. For shallow

swarms the fine spray always gives the higher efficiency, but is much inferior to the coarse spray for the attack of deep swarms. The efficiency of the fine spray depends much more critically upon the depth of the swarm than does that of the coarse spray.

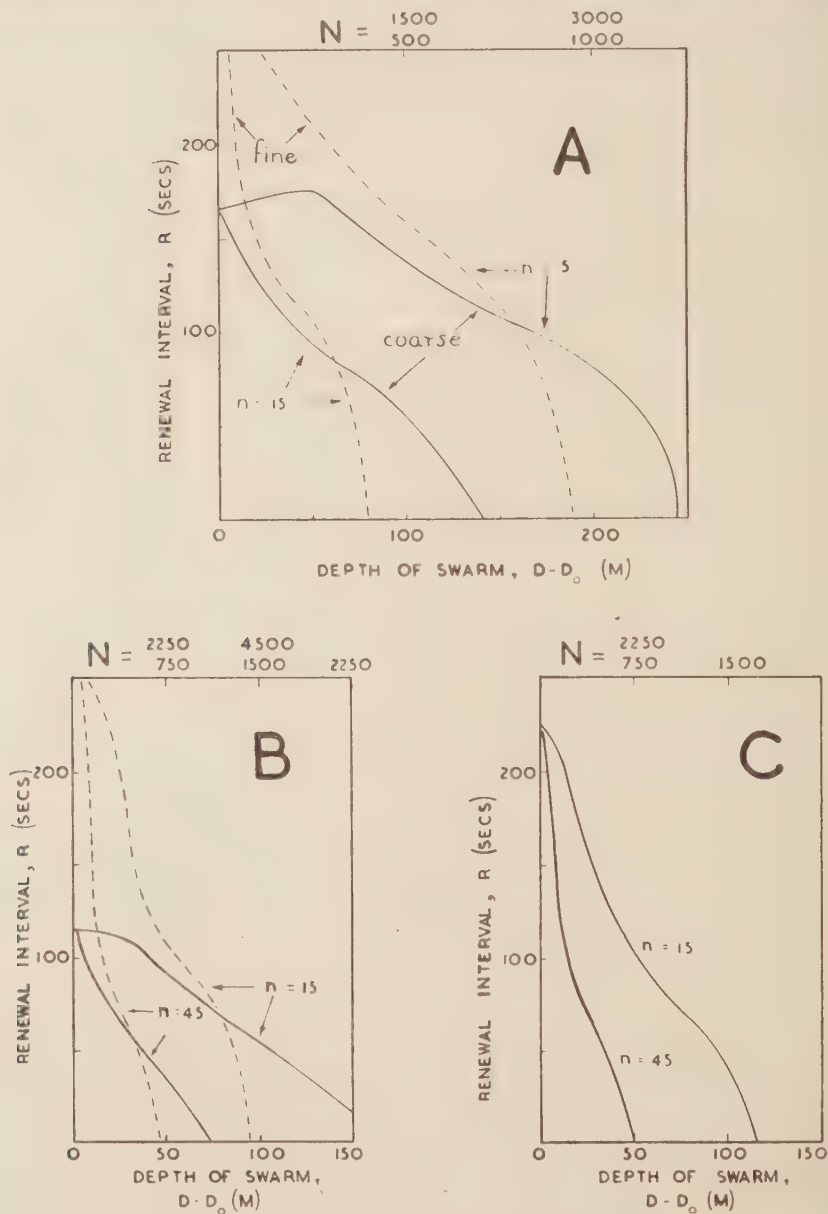


Fig. 15.—Renewal interval between curtain elements required to maintain a minimum dose of $150 \times 10^{-6}g.$ at the bottom of a swarm, as a function of swarm depth and area density when—

- A. $D_0 = 100$ m.
- B. $D_0 = 50$ m.
- C. $D_0 = 200$ m.

Weighing up the evidence of figs. 15 and 16, the coarse spray shows an appreciably greater flexibility and for this reason is to be preferred where conditions in the field may vary over a wide range. In the special case where it is certain that the swarms to be attacked will always be flat-topped, sparsely populated and shallow, the fine spray is the better choice.

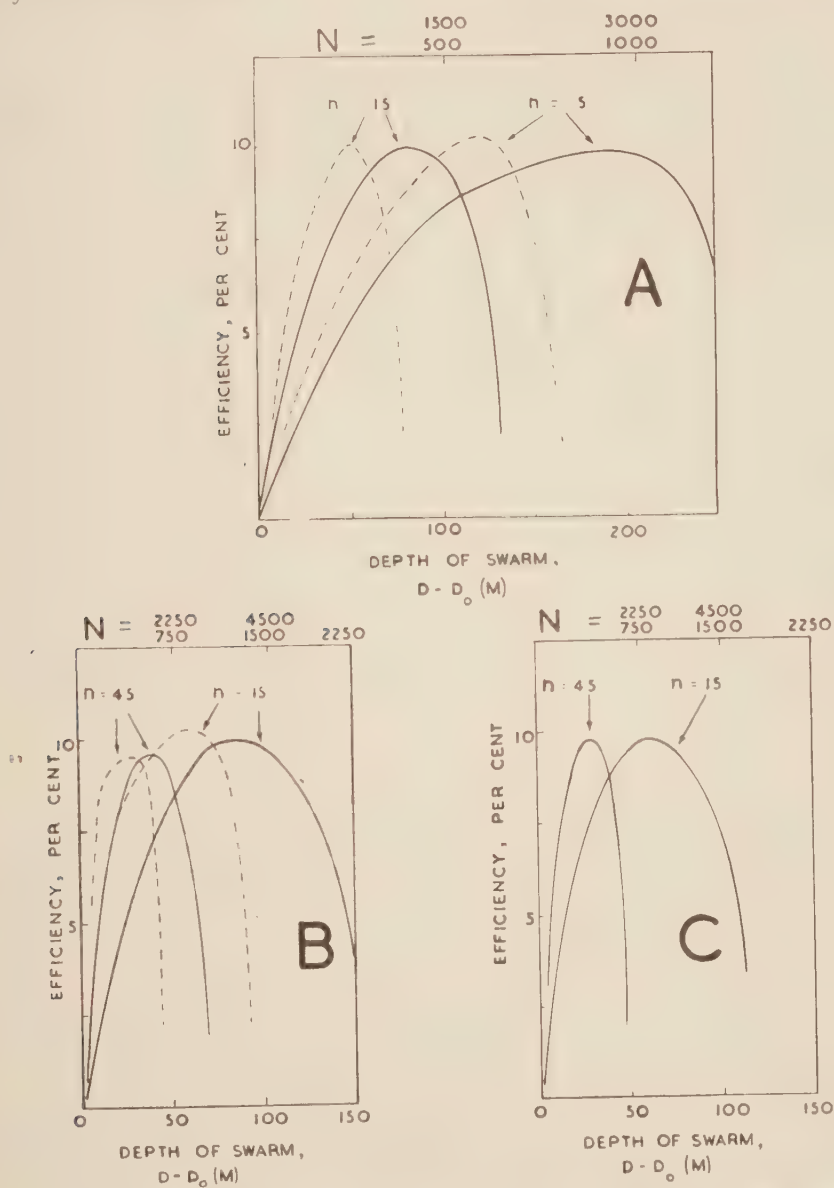


Fig. 16.—Efficiency of curtain maintaining a minimum dose of 150×10^{-6} g. at the bottom of a swarm, as a function of swarm depth and area density when—

- A. $D_0 = 100$ m.
- B. $D_0 = 50$ m.
- C. $D_0 = 200$ m.

Ideally, there is an optimum combination of droplet size distribution and spraying height, varying with swarm depth and density which will yield maximum efficiency on each occasion. Such complete flexibility in the field is difficult to obtain and it is therefore encouraging to find that a satisfactory performance over a fairly wide range of conditions can be obtained with a fixed droplet size distribution.

Quantity of Spray usefully applied.

The total quantity of spray deposited by a curtain on a swarm is greater than the efficiencies given in fig. 16 would suggest. In calculating these values the necessarily excessive dosage applied at the top of the swarm and the overlapping tails of previous elements were deliberately excluded. When these are taken into account and conditions are around the optimum in fig. 16, some 50 to 70 per cent. of the total weight of the element is deposited on the locusts. In any particular case, the total quantity deposited can be obtained from figs. 5 to 14, by multiplying the area of each zone by the mean of its contour values and summing the products for the whole of the diagram.

The numerical values obtained in fig. 16 for the efficiency of an operation depend to some extent upon the basic quantities used in the preceding calculations. Slightly higher efficiencies could have been obtained by a suitable choice of basic data. Practical efficiencies in the field are bound to fall short of values given here, but even if it should prove possible to apply the spray with an efficiency of only one per cent., this will still be much more efficient than most insecticide applications. The ultimate test of efficiency is, however, economic.

The numerical data given above are quantitatively correct on the information given in figs. 1 and 2, but clearly a good deal depends upon the exact form of the curve in fig. 2 for the equivalent area of the locust. Only one species was used for the experiments and it was necessary to assume, for lack of experimental data, that mortality is independent of droplet size and of where the droplets are deposited on the locust. The airspeed of a locust in a swarm may differ appreciably from the value taken here. For these reasons the numerical values given above may need revision as the results of further experimental work become available, but the principles they illustrate remain generally applicable.

Summary.

This paper deals with the aerial curtain method of spraying flying swarms of locusts with insecticide from aircraft.

Equations are developed for calculating the dosage deposited on locusts in single spraying runs under idealised conditions. Graphical methods of solving the equations are described together with a simple method for obtaining the summed effect when a number of spraying runs are carried out in a regular sequence to form an aerial curtain.

There is an optimum combination of spray droplet size distribution and spraying height according to the depth and density of the swarm, but good performance can be obtained with a single fixed droplet size distribution. A fine spray is recommended for the attack of flat-topped, shallow swarms, but a coarser spray is required to penetrate adequately into deeper and denser swarms.

The efficiency of application of the insecticide solution, calculated as the proportion of lethal doses actually applied to those potentially available, may be as high as 10 per cent., but varies rapidly with spray droplet size and swarm characteristics.

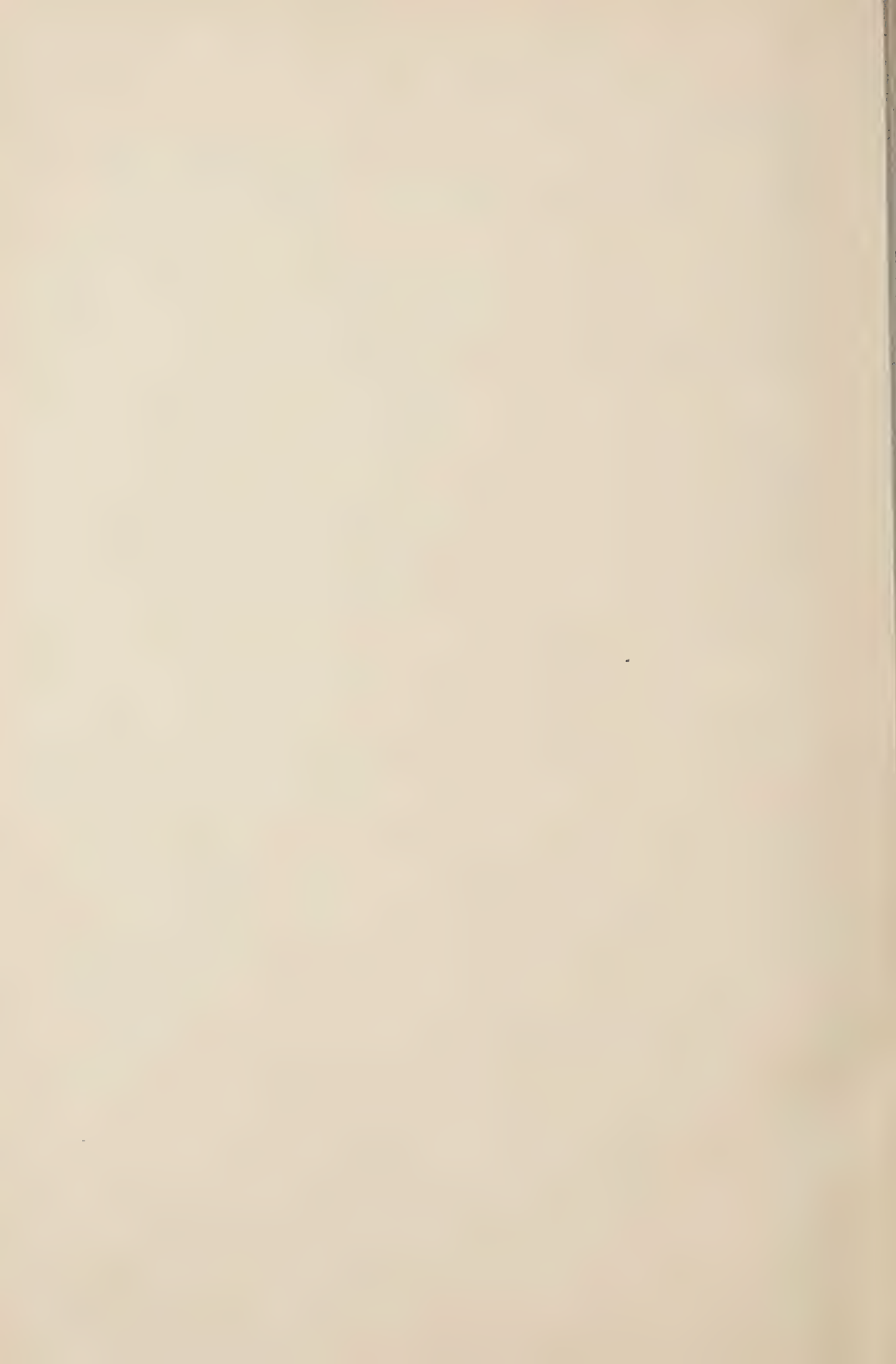
The numerical values given may need some modification in the light of further experimental and field data, especially in regard to locust airspeed in the swarm and the effect of droplet size on mortality.

Acknowledgements.

The author's thanks are due to Mr. W. L. Dennis, who was responsible for the experimental data quoted in fig. 2, to Dr. D. L. Gunn (Anti-Locust Research Centre, London) for a number of stimulating discussions, and to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

References.

- FUCHS, N. (1936). Velocity of fall of particles for which Stokes' Law no longer holds. [In Russian.]—Zh. tekhn. Fiz., **6**, pp. 709-711.
- GUNN, D. L., GRAHAM, J. F. & others. (1948). Aircraft spraying against the Desert Locust (*Schistocerca gregaria* Forskal) in Kenya, 1945.—Anti-Locust Bull., London, no. 4, 121 pp.
- GUNN, D. L., LEA, H. A. F. & others. (1948). Locust control by aircraft in Tanganyika.—153 pp. London, Anti-Locust Res. Cent. & Pretoria, Locust Contr. Res. Sect. Dep. Agric.
- GUNN, D. L., PERRY, F. C. & others. (1948). Behaviour of the Desert Locust (*Schistocerca gregaria* Forskal) in Kenya in relation to aircraft spraying.—Anti-Locust Bull., London, no. 3, 70 pp.
- KENNEDY, J. S., AINSWORTH, M. & TOMS, B. A. (1948). Laboratory studies on the spraying of locusts at rest and in flight.—Anti-Locust Bull., London, no. 2, 64 pp.
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THE SEASONAL INCIDENCE OF *IXODES RICINUS* (L.) ON CATTLE IN MID-WALES.

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The seasonal activity of *Ixodes ricinus* (L.) in Scotland has been described by Meek and Greig Smith (1896), Stockman (1916) and MacLeod (1932). According to these investigators the incidence of this tick on sheep follows a two-phase curve, a spring phase that terminates in late May or early June and an autumn phase of lower intensity during September and October, the infestation being relatively low during the summer and absent during the winter. MacLeod (1936) advanced a theory, based primarily on laboratory work, that this periodicity was related to temperature conditions, "the ticks forsaking the tips of vegetation above and below a certain range of temperature". As a result of more extensive field investigation MacLeod (1939) found that "the limits of air temperature corresponding to active tick infestation are 45 and 65 F., average weekly maximum". Hendrick, Moore and Morison (1938) showed that in N.E. Scotland tick activity on sheep was continuous throughout the year with maximum activity in July. MacLeod (1939) suggests that this incidence curve may be due to abnormal husbandry conditions practised in that region.

Milne (1945a) has established the occurrence of the two-phase curve of tick activity on sheep in North England. The theory of temperature control (MacLeod, 1939) does not apply to activity in this region. Milne (1945a) found that about half the normal annual female activity at Crag, S.W. Cumberland, occurred at temperatures of 60 to 70°F.

The seasonal incidence of the tick on cattle has not been so extensively studied as on sheep. Edwards and Arthur (1947) report the normal spring and autumn peaks of activity on cattle in South Wales. In mid-Wales, however, they claim the existence of a single peak of activity of maximum intensity in late August and September. Arthur (1948) supports this finding with observations made at Barmouth, and Llanuwchllyn in Merionethshire and at Tregaron in Cardiganshire.

In the present investigation a detailed study has been conducted of the incidence of tick on cattle under various husbandry methods in N.W. Cardiganshire.

The seasonal Incidence on Cattle on selected Farms.

The method used for counting the ticks on cattle involved a total count of the number of females attached on the fore- and hindquarters of the animal. To facilitate counting, the forequarters were divided into the head, dewlap and foreleg regions and the hindquarters into the udder, dewlap, hindlegs and escutcheon. The infestation of sheep was recorded by the technique described by Milne (1945b), and entailed counting the female ticks on the head, axillary and inguinal regions of the body. The infestation of cattle and sheep was recorded at seven to 11 day intervals during the period of observation.

The farms included in the investigation were carefully chosen so as to be representative of the various husbandry methods practised in the region. A total of nine farms was visited during 1947 and 1948 and, on four of these, observations were made throughout the two-year period, with changes in the normal stocking

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of the infested pastures on each farm during the second year. The distribution of the farms in the region is shown in fig. 1.

(a) Bryndderwen.

This farm is situated in the Dolybont district (fig. 1) and is essentially a small holding comprising 10 acres of grassland. The major part consists of a 7-acre meadow bordering the river Leri. This meadow is poorly drained and the existing *Agrostis* pastures have been colonised by rush (*Juncus* spp.) and, in the drier parts, by bracken-fern (*Pteris aquilina*). From March to December three Welsh Black cattle grazed the heavily tick-infested meadow except for occasional visits to the remaining three acres of *Agrostis* with ryegrass pastures.

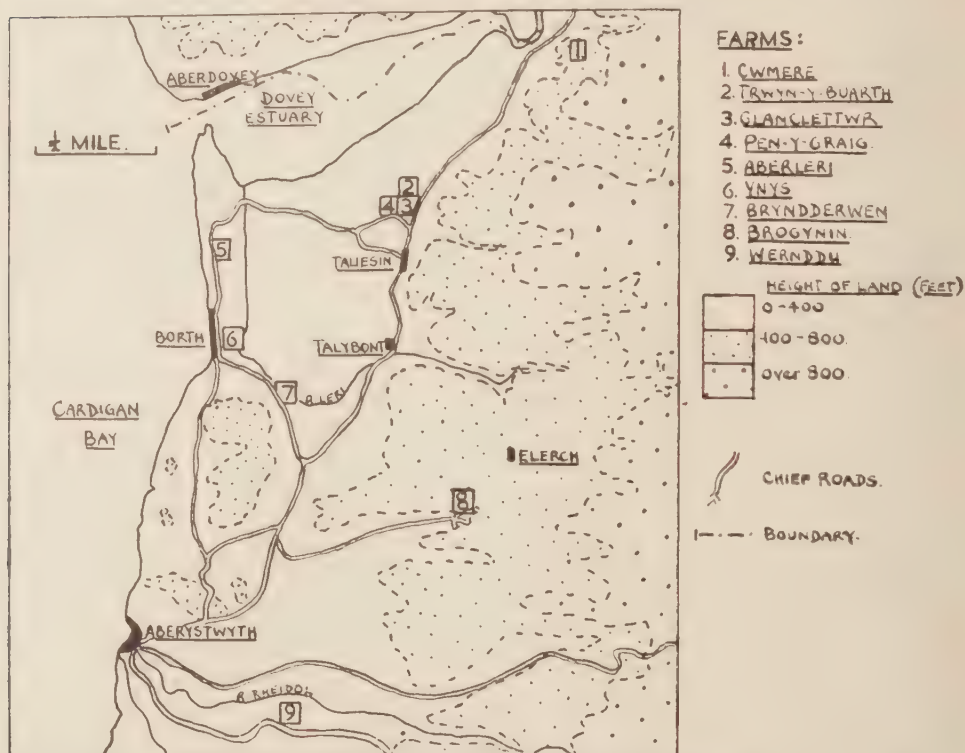


Fig. 1.—The distribution of nine farms included in the investigation of the seasonal incidence of *Ixodes ricinus* in N.W. Cardiganshire.

In 1947 the cattle were turned out on 27th March and the first examination of the animals five days later recorded an average infestation of 45.3 ticks per cow. The infestation increased to a peak of 301.0 on 22nd April (fig. 2) then gradually decreased to 0.7 ticks per cow on 29th July. A second phase of tick activity commenced on 5th August culminating in a peak infestation of 206.7 ticks per cow on 16th September. The infestation had decreased to 0.7 ticks per beast when the animals were taken off the grazings in early December. In 1948 the cattle were exposed to infestation on 3rd March and a light infestation of 10.0 ticks per cow was recorded a week later. The spring peak of activity occurred on 27th April with 222.0 ticks per animal. The autumn peak of activity was registered on 14th September with 229.0 ticks per cow. Observations were discontinued after 5th November.

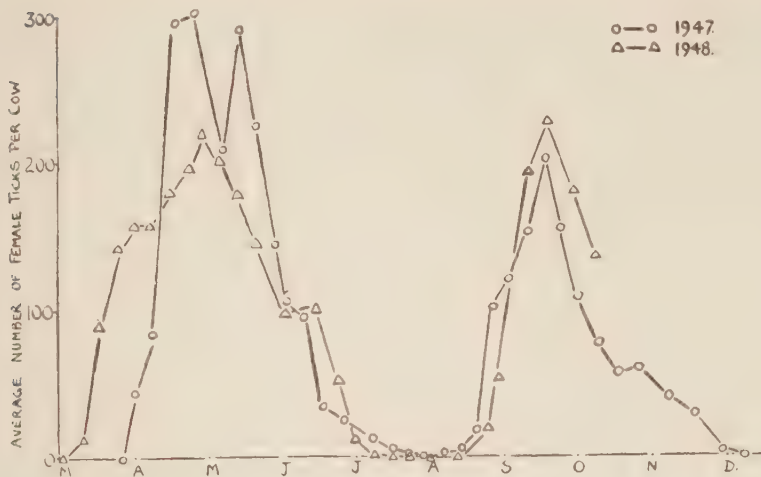


Fig. 2.

A comparison of the infestation curves for 1947 and 1948 shows a very marked difference between the degree of infestation of the cattle during the spring phase of activity. The later stocking of the meadow in 1947 appears to have resulted in a higher infestation of the stock than that which occurred under "continuous" stocking in 1948. The effect gradually diminished until there was no apparent difference in the infestation curves during June. The higher infestation recorded in 1947 may also be due, in part, to the normal yearly variations in the intensity of tick activity (Milne 1945a). The delay in stocking, however, did not prolong the female tick activity into the normal off-season. This agrees with the findings of Milne (1945b) for sheep in North England.

(b) Ynys.

The tick infested pastures on this farm are almost entirely confined to a large expanse of rough land bordering the Borth Bog (Cors Fochno). The infested pastures formed only about 20 per cent. of the total available grazing land and the herd consisted of 20 Welsh Black cattle; there were no sheep on the holding. The activity curve (fig. 3) is based on counts of attached female ticks on the same ten cows at intervals from 7th May to 20th November 1947.

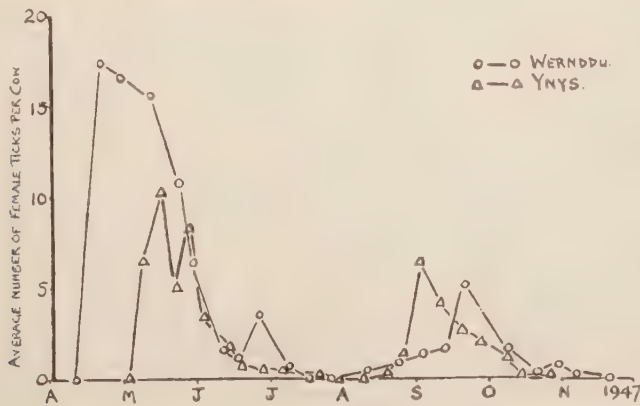


Fig. 3.

The herd was turned out to the infested grazings on 17th April, and a peak infestation of 10.2 ticks per cow was observed a fortnight later. The infestation gradually declined to 1.9 ticks per beast on 9th June and subsequently fluctuated at a low level until the commencement of the "autumn" phase of activity on 26th August. From September to November the cattle were turned on to the infested grazing at infrequent intervals. The "autumn" peak of activity occurred on 1st September with an average of 6.3 ticks. The herd was tick-free on 20th November.

The low infestation recorded on this farm was probably due to the infested pastures forming only a small proportion of the available grazing land.

(c) Wernddu.

Tick infested grazings at Wernddu were restricted to an 8-acre rush-ridden meadow, the remaining 45 acres of grassland forming good tick-free grazing land. In spring the meadow was heavily stocked but after the hay harvest other pastures became available with the result that the cattle only spent short periods on the meadow during late summer and autumn. The herd comprised 24 cattle of various breeds. Tick counts were made on the same ten cattle during 1947.

Twelve days after exposure the cattle recorded an average infestation of 17.3 ticks per animal (fig. 3). As on the other farms visited a gradual decrease in the infestation followed. The "autumn" phase of activity was of low intensity with a peak infestation of 5.2 ticks per cow on 17th September.

The delayed stocking of the infested pastures has resulted in an immediate heavy infestation of the cattle. The curve, however, is typical of the descending arm of the normal activity curve.

(d) Aberleri.

This farm is situated on the western margin of the Borth Bog. Large-scale drainage of a number of the fields between 1940 and 1945 substantially decreased the area of rough grazing on the holding. Eight cattle from a herd of 25 animals were examined during 1947 and 1948.

In 1947 the cattle were turned out on 18th April and carried an average of 6.8 ticks per beast, when first examined on 2nd May (fig. 4). The infestation increased to 20.9 ticks per cow on 22nd May, but a very low infestation was evident throughout June, July and August. In the "autumn" period of activity a peak infestation of 7.9 ticks per cow was recorded on 8th September. In 1948 it was difficult to determine the exact period of maximum activity in the spring owing to the movement of the

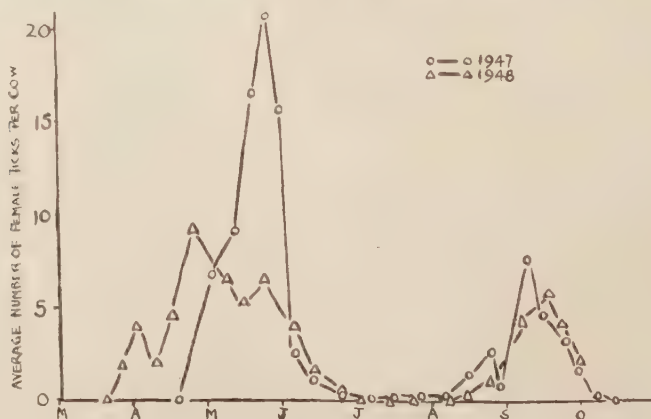


Fig. 4.

cattle from one field to another. Under these conditions a peak of 9.4 ticks per cow on 22nd April was followed by a "secondary" peak on 20th May. This phenomenon, coupled with the availability of alternative tick-free grazings in late summer, complicated the timing of the "autumn" peak of activity. The effect of the earlier stocking of the infested pasture on the incidence of the tick was similar to that at Bryndderwen (p. 461).

(e) Brogynin.

The heaviest stocking of the tick infested pastures at Brogynin occurred in late summer and autumn and not in the spring and early summer as at Ynys, Werddu and Aberleri farms. The infested grazing was characteristic of lowland tick-infested farms in that it was confined to a small acreage of the available grassland, a 13-acre meadow invaded by bracken-fern and rush. The remainder of the grazing land was situated at a higher elevation, and was practically tick-free. The farmstock consisted of 20 breeding ewes, that were never turned onto the meadow, and 12 dairy cattle. The infestation of the same cattle was recorded in 1947 and 1948.

During 1947 the herd was turned out on 15th April and was confined to the lightly infested fields around the farm buildings until 10th May. On that date they were transferred to the infested meadow where they remained, during the daytime, until the beginning of July. The cattle made infrequent visits to this grazing during July, but in August, September and October they only left the meadow for milking. After 21st October this grazing became excessively wet and the cattle were moved to the pastures at higher elevations. The rotation of grazing on the farm had a definite effect on the seasonal incidence of the tick (fig. 5). In spring the peak infestation occurred within five days of their being transferred to the infested meadow. A month later the infestation had decreased to 0.8 ticks per cow. Female ticks were only occasionally found on the animals from 25th June to 7th August. The "autumn" phase of activity was evident on 18th August and reached its maximum of 74.0 ticks per cow on 11th September. The infestation had decreased to 29.6 ticks per cow when the animals were removed from the infested pastures on 15th October.

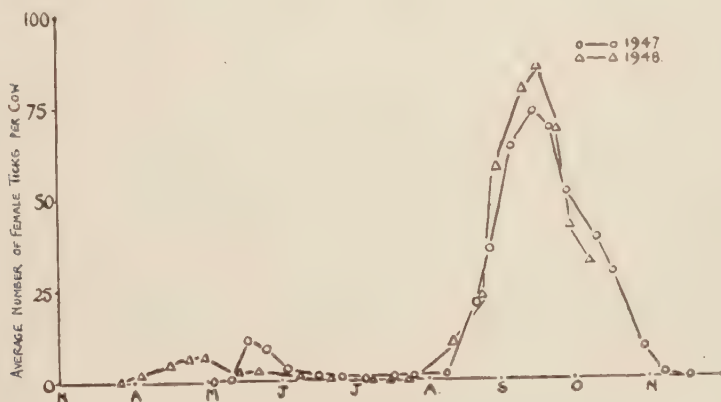


Fig. 5.

The earlier stocking of the meadow in 1948 further demonstrated the low population of spring ticks due to the restricted grazing. In the autumn, as is evident from the heavier infestation of the cattle, continuous stocking permits of the development of a far greater number of ticks.

(f) Cwmere.

The grazing of infested pastures on this holding is typical of the marginal farms of Cardiganshire. The major part of the grazing land lies at elevations between 600 ft. and 1,000 ft. A survey of the extent of tick-ridden grassland on the farm showed that 80 per cent. of the available grazing land was infested. Fescue-*Agrostis* pastures invaded by bracken-fern and, at higher elevations, mountain fescue with *Nardus*, formed the infested grazings. The farmstock comprised over 1,500 sheep, 15 to 20 dairy cattle and a variable number of store cattle. The sheep were confined to the hill grazings except for about 100 ewes which were brought down to the sheltered fields in the fescue-*Agrostis* belt, for lambing. Dairy cattle grazed the fescue-*Agrostis* pastures ("the fridd") whilst the store cattle were restricted to the hill grazings. The same five dairy cows were examined for ticks in 1947 and 1948.

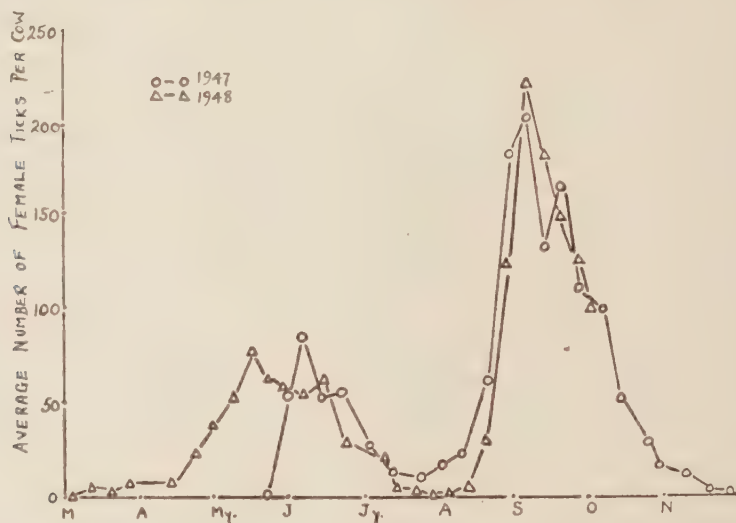


Fig. 6.

In 1947 the dairy cattle were turned on the "fridd" on the 16th May and a fortnight later carried an average infestation of 55.0 ticks per beast (fig. 6). The peak infestation was recorded on 6th June, and subsequently the infestation decreased to a minimum of 11.6 ticks per cow, on 25th July. A second phase of activity commenced on 1st August reaching a peak of 203.2 ticks per animal on 3rd September and declining to below 2 ticks per animal at the end of November when the cattle were taken off the grazings. The following year observations began on 10th March when the animals were grazing on well-managed fields around the farmstead. The infestation of the cattle whilst on these pastures was light and had only reached an average of 8.4 ticks per animal when the cattle were transferred to the "fridd" on 19th April. The cattle recorded heavier infestations on the "fridd", reaching a peak of 79.2 ticks per cow on 14th May. A relatively heavy infestation was maintained until 6th July, but from 13th July to 20th August only occasional female ticks were found on the cattle. The "autumn" phase of activity was approximately of the same intensity as in 1947.

The lower incidence on cattle in spring as opposed to the heavy infestation recorded in late summer and autumn was probably due to a large proportion of the unfed "spring" ticks becoming replete after feeding on the ewes and lambs. Tick

counts on five ewes and five lambs, restricted to 5 acres of the "fridd", indicated that large numbers of female ticks were fed by the sheep before the cattle were turned to the rough grazing (Table I). Cattle and sheep grazed the infested pastures from 19th April to 23rd May when the sheep were transferred to hill grazings. These pastures were grazed by cattle only in the summer and autumn.

TABLE I.

The incidence of female ticks on ewes, lambs and dairy cattle on similar grazings at Cwmere.

Date 1948	Average number of female ticks on		
	Ewes (5)	Lambs (5)	Dairy Cattle (5)
27th March	11.2	—	—
2nd April	12.6	—	—
8th "	18.0	—	—
14th "	23.4	—	—
21st "	32.6	19.2	20.2
28th "	18.0	34.8	—
30th "	—	—	29.0
7th May	15.8	25.6	50.2
14th "	12.2	23.8	79.2
21st "	9.6	22.8	63.4
25th "	—	—	60.4

The same ewes, lambs and cattle used for tick counts.

(g) Trwyn-y-Buarth, Pen-y-graig and Glanclettwr.

These three farms are situated on the eastern margin of the Borth Bog. The tick-infested pastures on all three holdings are almost entirely confined to the rough grazings provided by the marshland. The utilisation of the marshland varied on each farm and in 1948 observations were made on four cattle at Trwyn-y-Buarth and at Pen-y-graig and five cattle at Glanclettwr.

At Trwyn-y-Buarth the grazing land consisted of three rush-ridden fields situated on the margin of the bog. The herd grazed these pastures throughout the year. Pen-y-graig, in addition to a large expanse of marshland, had three well-managed fields near the farmstead which were very lightly infested. The cattle were restricted to the lightly tick-infested pastures during March and the beginning of April when they were transferred to the marshland, the fields being rested for hay. From July to November the cattle grazed the well-managed fields and only visited the marshland for short periods during the daytime. The rough grazings at Glanclettwr formed only a small proportion of the available grazing land. The tick-infested pastures were stocked in the daytime, from late April until November.

The seasonal incidence of the tick on these farms is shown in fig. 7. The early and continuous exposure of the cattle to the tick at Trwyn-y-Buarth results in the normal bimodal curve with maximum activity in April and September (fig. 7). At Pen-y-graig light infestations were recorded when the cattle were on the fields near the farmstead but on transference to the marshland the infestation of cattle increased to a peak of 161.0 ticks per cow on 10th May and continued at a lower level throughout July; the autumn peak was substantially lower. The late stocking of infested pastures at Glanclettwr caused the period of maximum infestation to be

delayed until 19th May but only occasional ticks were attached on the cattle during July. Autumn activity resembled that on the other two farms.

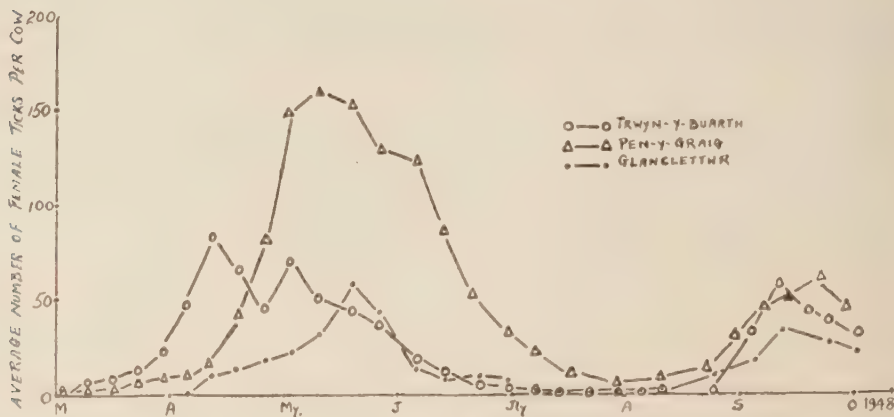


Fig. 7.

Conclusions.

In N.W. Cardiganshire the seasonal incidence of female ticks on cattle available to the parasite during the whole period of activity showed two maxima, one in April and the other in September. Activity in autumn was generally of a lower intensity than in spring. There were marked deviations from the normal infestation curve as a result of grazing practises. The three factors chiefly responsible were :—

1. The delayed stocking of infested pastures ;
2. The rate of stocking of infested pastures ;
3. The presence of alternative hosts on the same grazing.

When stocking was delayed an initial heavy infestation of the cattle occurred, e.g., Bryndderwen 1947, Aberleri 1947 and Wernddu. This effect gradually diminished and, except at Pen-y-graig where there was evidence of a higher than normal infestation in July, the infestation curve eventually converged with the normal activity curve. It was not possible to ascertain from the present investigation whether the peak infestation from delayed stocking was higher than that which would have occurred under continuous stocking. A comparison of the degree of infestation of the same cattle in different years is not valid since Milne (1945a) has shown that peak height varies from year to year under continuous stocking.

The relative area of available grazing land colonised by the tick and the time the cattle and other domestic hosts spend on the infested pastures controlled the degree of infestation of the animals. Infrequent visits by cattle to the grazing limited the build up of the tick population and light infestations were recorded. The maximum grazing of infested pastures in autumn resulted in heavier infestations of the cattle during that period than in spring (*cf.* fig. 5).

The presence of alternative hosts causes a marked decrease in the infestation of the major host on the same grazing. For example at Cwmere farm the repletion of a large proportion of the unfed tick population by sheep before the arrival of cattle on the grazing resulted in a lower infestation of the cattle in spring than in autumn when they grazed the pastures alone.

Discussion.

The present investigations in the seasonal incidence of *I. ricinus* on cattle in N.W. Cardiganshire has confirmed and expanded the conclusions drawn by Milne (1945a & b) from similar work on sheep in North England.

The unimodal type of activity reported by Arthur (1948) to be characteristic of Merionethshire, and Cardiganshire is not true of N. Cardiganshire. It was based on occasional visits to numerous farms in these regions and on the examination of cattle at varying intervals from May to October on three farmsteads at Barmouth, Bala and Tregaron. These farms are in the marginal zone. From personal experience the writer is doubtful whether conclusions drawn from an occasional examination of the farmstock and from data obtained from farmers can be regarded as wholly reliable. The seasonal incidence curve recorded by Arthur (1948) as being typical of unimodal activity on cattle shows a low infestation of the animals from May to early June followed by increased activity culminating in a peak of over 120 ticks per beast in late August or early September. The only variation between this curve and the one recorded by the writer at Cwmere (fig. 6) is in the extent of tick activity during May and June. This difference is possibly due to the fact that only two counts were made during the whole of May and June on each of the three farms visited by Arthur as opposed to approximately weekly counts made of the writer. At Barmouth where the two counts were made within three weeks of each other a low peak infestation is evident in mid-June. On the other two farms the counts were separated by at least four weeks. This interval is sufficient for the activity of a small population of spring ticks to be unobserved (fig. 3). The lowering of the infestation of cattle in the spring is a characteristic feature of marginal farms as a result of the repletion of large numbers of unfed ticks by ewes brought down from the hills for lambing.

Milne (1945b) suggested that "farmstock can have the run of infested land in the normal off seasons of activity without danger of infestation even if the land has not been grazed in the seasons of activity". This does not appear to be practicable for cattle in mid-Wales. Generally dairy herds are only kept out of doors from March to November and during this period, on heavily infested farms, infestations of below 20 ticks per cow only occur for about eight weeks (mid-July to mid-August). Control measures must therefore entail the improvement of the infested pastures or the treatment of farmstock with acaricidal compounds. On lowland farms where the infested land is usually restricted to ill-drained meadows, pasture improvement may be practicable. The improvement of hill grazing is more difficult and often not economic (Milne, 1948). Under these conditions the farmer must concentrate on the treatment of this farmstock.

Summary.

An investigation of the seasonal incidence of *I. ricinus* on cattle in N.W. Cardiganshire showed that there are normally two peaks of activity, one occurring in spring and the other in autumn. The infestation curve varied considerably from farm to farm according to the husbandry methods practised. Delayed stocking of infested pastures caused an initial higher infestation of the cattle but, except on one farm, did not prolong the infestation beyond the normal period of tick activity. A lower infestation in spring than in autumn occurred on two farms. This resulted from either the lighter stocking of the infested grazings in spring than in autumn or the partial exhaustion of unfed tick population by sheep grazing with the cattle during the spring only.

There was no evidence for the occurrence of a unimodal curve of tick activity in the region studied.

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References.

- ARTHUR, D. R. (1948). *Bull. ent. Res.*, **39**, pp. 321-337.
EDWARDS, E. E. & ARTHUR, D. R. (1947). *Parasitology*, **38**, pp. 72-85.
HENDRICK, J., MOORE, Walter, and MORISON, G. D. (1938). *Nature*, **141**, p. 648.
MACLEOD, J. (1932). *Parasitology*, **24**, pp. 382-400.
MACLEOD, J. (1936). *Ibid.*, **28**, pp. 295-319.
MACLEOD, J. (1939). *Bull. ent. Res.*, **30**, pp. 103-118.
MEEK, A. & SMITH, R. Greig (1896). *Veterinarian*, **69**, pp. 269-276, 363-368.
MILNE, A. (1945*a*). *Parasitology*, **36**, pp. 142-152.
MILNE, A. (1945*b*). *Ibid.*, **36**, pp. 153-157.
MILNE, A. (1948). *Ann. appl. Biol.*, **35**, pp. 369-378.
STOCKMAN, Sir S. (1916). *J. comp. Path.*, **29**, pp. 244-264.
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THE DISTRIBUTION AND ECONOMIC IMPORTANCE OF *IXODES RICINUS* (L.) IN WALES AND THE WELSH BORDER COUNTIES WITH SPECIAL REFERENCE TO N.W. CARDIGANSHIRE.

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Introduction.

Ixodes ricinus (L.) (Acarina : Ixodoidea) is an ectoparasite of domestic animals in Britain. According to MacLeod (1939) this tick is widely distributed on hill grazings in Scotland and northern England and is fairly common in Ireland, North Wales and south-west England. *I. ricinus* is a three-host tick : the larva, nymph and female spending, respectively, three to four, four to five, seven to 12 days on the host out of a total period of three years required for the completion of the life-cycle (Campbell, 1946). The remainder of the life-cycle is spent on the ground. Under laboratory conditions, MacLeod (1935) found that the dominant climatic requirement of unfed stages of the tick was a high degree of humidity. This environmental requirement was also inferred by Milne (1944) to be the limiting factor in the distribution of the tick on cattle and sheep in N.W. Northumberland. Tick-infested pastures in that region were restricted to rough hill and moorland grazings in which the dominant plant was one of the rough grasses (*Molinia*, *Nardus*, *Agrostis*, *Aira*), bracken-fern or heather. These plants, when they wither, form a layer of moisture-bearing debris over the soil and provide the permanent moist microclimate essential for the survival of the tick.

I. ricinus is responsible for transmission of a number of important diseases of sheep and cattle. MacLeod & Gordon (1932), in Scotland, conclusively showed that the virus of louping ill, an encephalomyelitis of sheep, was transmitted by the tick. Among native acclimatised stock the mortality rate from this disease is below 10 per cent. of those sheep susceptible, *i.e.*, lambs and hogs undergoing their first "tick season". A mortality rate of over 50 per cent. may occur when non-acclimatised stock, of any age, is introduced to infested pastures (MacLeod, 1939). Cattle were also susceptible to louping ill often with fatal results. MacLeod & Gordon (1933) also demonstrated the transmission of tick-borne fever by *I. ricinus*. This disease is closely related to louping ill but in the absence of secondary infections seldom terminates fatally. These two tick-borne diseases are widespread in Scotland and northern England. There is no record of their occurrence in the other tick-infested regions of Britain.

Lambs grazing heavily tick-infested pastures in Scotland, northern England and North Wales often contract a pyaemic condition characterised by multiple abscess formation in the skin, internal organs and the joints. The regular association of the disease with tick-infestation of the lambs has led to the belief that it results either from the contamination of tick-bite lesions or from the inoculation of pyogenic organisms by the tick itself. This condition has been termed tick pyaemia. Lyle Stewart (1941), in a survey of tick-borne diseases in northern England, states that losses from tick pyaemia in that region overshadow those from louping ill and tick-borne fever.

The chief tick-borne disease of cattle in Britain is piroplasmosis or redwater fever. According to MacLeod (1939) the disease, characterised by a haemoglobinurea,

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is endemic in Ireland, Wales and Scotland and in the northern and south-western counties of England. In Britain, the causal agent is generally considered to be the protozoan parasite, *Babesia bovis*, but details of its life-history are not known. Cattle indigenous to redwater areas are generally resistant although immunity is not absolute (Nat. Med. Ass. Gt. Brit., 1945).

In the present study an attempt has been made to define the distribution and economic importance of *I. ricinus* in Wales and the Welsh border counties. A detailed survey has also been conducted of the distribution of the tick in relation to vegetation and farming conditions in N.W. Cardiganshire. These observations were made in 1947 and 1948.

Species of Tick infesting Farmstock in Wales.

Previous work on *I. ricinus* in North Wales by Walton (1927) and in South Wales by Edwards & Arthur (1947) has demonstrated the widespread occurrence of this species in Wales. During the present investigation on 214 tick-infested farms in the counties of Caernarvon, Merioneth, Montgomery, Cardigan and Carmarthen, the two species of ticks, *I. ricinus* and *Dermacentor reticulatus* (F.) were encountered on cattle and sheep. *I. ricinus* is the more common and occurred on all the farms visited. *D. reticulatus* was restricted to two neighbouring farms near the Borth Bog, N. Cardiganshire. This appears to be the first record of *D. reticulatus* infesting cattle and sheep in Wales.

MacLeod (1932) recorded the occurrence of *Ixodes hexagonus* Leach, on sheep in the Ettrick district of Scotland and Milne (1944) found sheep-dogs infested with *Ixodes canisuga* Johnston in Northumberland. These two species have not been noted on sheep in the region examined by the writer, but two vixens, *Vulpes vulpes crucifera* (Bechstein), trapped in the Aberystwyth district carried heavy infestations of both nymphal and adult stages of *I. hexagonus* and *I. canisuga*.

Throughout the remainder of this work "ticks" will refer to *I. ricinus* only.

The Distribution and economic Importance of the Tick in N.W. Cardiganshire.

(i) Description of the region.

The region shown in fig. 1 represents approximately 95 sq. miles of land in N.W. Cardiganshire ranging from sea level to 1,700 ft., with the greater part below 1,000 ft. The Coastal Plateau, which at its seaward limit stands at 400 ft. and at its margin rises to 900 ft., is separated by a marked shelf. This shelf, according to Jones (1911) marks the position of an ancient coastline. In this region the High Plateau occupies the eastern portion of fig. 1. The north-western area of the map is occupied by a large expanse of low-lying, poorly drained ground known as the Borth Bog (Cors Fochno).

The basal geological formations are composed of Ordovician and Silurian strata. Silurian rocks predominate and consist of grits, flags and shales. The former, the Aberystwyth Grits, form a continuous tract of elevated country, approximately 2 miles wide running southwards along the coast from Borth. The central area consists of flags and soft shales, passing in the N.E. and E. to grits and shales of Ordovician age. There is no limestone anywhere in the region and the soils tend to be acid and podzolised.

The Coastal Plateau is deeply cut by four rivers, the Ystwyth, Rheidol, Clarach and Leri all of which have a westerly trend and occupy U-shaped valleys showing the characteristic features of glaciation. Agricultural land in the lower reaches of the rivers is liable to periodic flooding.

Climatically the region is in the main mild and moist which is characteristic for the coastal areas of Wales. July isotherms range from 61 F. near the coast to 58°F. in the elevated regions, and January temperatures are in the region of 40°F. (E. G. Bowen, unpublished). Rainfall on the High Plateau is 60 ins. per annum and decreases to 40 ins. towards the sea.

(ii) *Technique.*

Before commencing the survey in N.W. Cardiganshire, a preliminary study was made, in 1947, of the seasonal incidence of the tick on cattle and sheep in the region

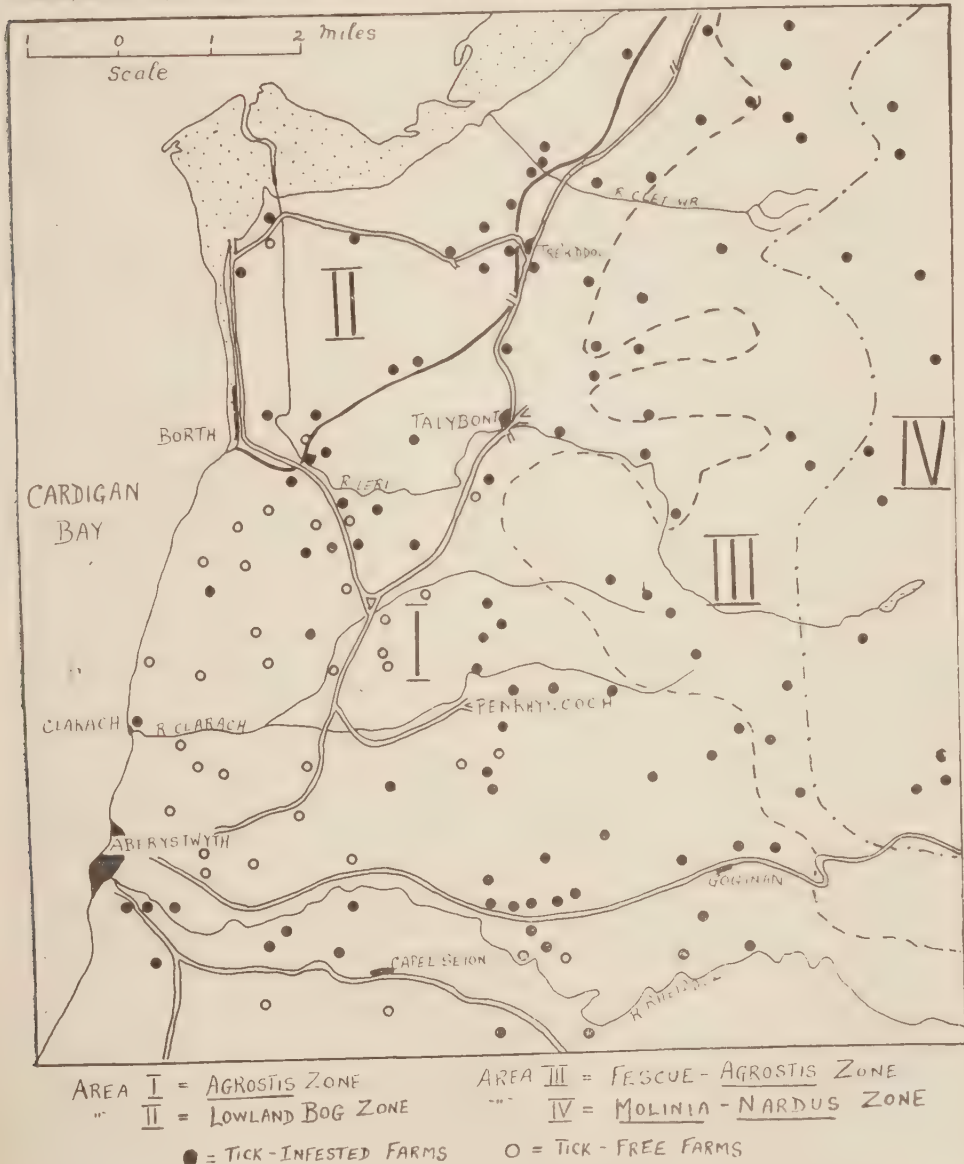


Fig. 1.—The distribution of *Ixodes ricinus* in N.W. Cardiganshire.

(Evans, 1950). It was possible from the data obtained to define the periods of tick activity and to compile seasonal activity curves on cattle under existing conditions. Tick activity on cattle and sheep in this region, is chiefly confined to two periods of the year, March to June and September to November. Delayed stocking of the infested pastures may limit the infestation of the domestic host to May and June and to September and October so that a true picture of the presence or absence of the tick on farmstock, on any farm taken at random, can only be obtained by examining the animals during those four months.

In the survey of 1948 the distribution of *I. ricinus* was based on the examination of either cattle or sheep or both on 148 steadings. Field data were recorded on a 1-inch-to-the-mile O.S. map. The number of female ticks attached on the cattle on these steadings was counted and with the aid of the seasonal incidence curves it was possible to estimate the peak infestation of the herd. The peak infestations were classified into three grades, *i.e.*, below 50 ticks per cow, 50 to 100 and over 100 ticks per cow. Hill cattle and sheep were examined when the animals could be penned with little inconvenience to the farmer.

The division of the region into the four major grassland zones shown in fig. 1 is based on a survey of the grasslands of Wales conducted by Davies (1936). In addition, a detailed field-to-field survey of the pastures of approximately 500 acres of farmland in the *Agrostis* zone and in the fescue-*Agrostis* zone was made by the writer. The dominant pasture-type or association of individual fields was plotted on 25-to-the-inch O.S. maps (scale $\frac{1}{2,534}$). The tick infestation of the pastures

was ascertained by the examination of the cattle and sheep on the grazings during May or September. The presence of ticks on the animals was only taken as indicative of the infestation of the pasture if the animals had been confined therein for a minimum period of 14 days. This period was sufficient to ensure that female ticks which might have attached to the animals on other grazings had engorged and dropped off; female ticks engorge in seven to 11 days on sheep (MacLeod, 1932) and within the same period on cattle (Edwards & Arthur, 1947). In the absence of animals on the pasture, four clean sheep were placed on the grazing and examined four days later.

The occurrence of bovine piroplasmosis on steadings in the region was obtained from interviews with farmers, and from the records of the treatment of the disease by veterinary practitioners. The distribution of tick pyaemia is also based on interviews with farmers in the district.

(iii) *Distribution of the tick in relation to vegetation.*

Visits were made to 148 farms, distributed as shown in fig. 1, of which 110 reported tick infestations of cattle or sheep. The distribution of tick-infested farms in relation to the grassland zone and the estimated peak infestation of the cattle on these steadings is given in Table I.

It is evident from Table I that there are significant differences in the proportion of tick-infested to tick-free farms in these grassland zones. The nature and extent of the tick-infested pastures in the four grassland zones are discussed below.

(a) *Agrostis* zone.

The *Agrostis* zone covers the grassland at elevations below 600 ft. The dominant grassland is composed of *Agrostis* pastures, which according to a transect of approximately 1,920 acres of land made in this zone by Davies (1936), formed 65 per cent. of the total area examined. *Agrostis* pastures in addition to the dominant *Agrostis* spp. (chiefly *A. tenuis*, *A. stolonifera* and *A. canina*) usually contain fog (*Holcus lanatus*), sweet vernal (*Anthoxanthum odoratum*) and white clover (*Trifolium*

TABLE I.

The distribution of tick-infested farms in the grassland zones of N.W. Cardiganshire with the estimated peak infestation of cattle on these farms.

Grassland Zone	No. of farms visited	No. of farms tick-infested	Percentage of farms tick-infested	No. of farms with the following peak infestation of cattle.		
				Below 50 ticks/cow	50 to 100 ticks/cow	over 100 ticks/cow
<i>Agrostis</i> Zone ...	102	66	64	56	7	3
Lowland Bog Zone ...	19	17	84	6	10	1
Fescue- <i>Agrostis</i> Zone...	17	17	100	2	10	5
<i>Molinia-Nardus</i> Zone	10*	10	100*	0	2	1

* Cattle available for examination on three farms only although on the remaining seven farms ticks were recorded on sheep.

repens) in varying abundance. On poorly drained and ill-managed land the *Agrostis* pastures are invariably invaded by rush (*Juncus* spp.). At higher elevations in the zone, poor soil and exposure result in a deterioration of the grasslands which are predominantly fescue-*Agrostis* pastures. These pastures are often colonised by bracken-fern (*Pteris aquilina*) and gorse (*Ulex* spp.). The *Agrostis* zone is extensively cultivated and includes approximately 70 per cent. of the farms in N.W. Cardiganshire.

The tick-infested farms in this zone may be seen from fig. 1 to be evenly distributed. The predominance of tick-free steadings along the coastal belt coincides with the outcrop of Aberystwyth Grits which form a region of excellent natural drainage. Tick-infested grazings that do occur on this outcrop are confined to isolated tracts of rough pasture resulting from poor management rather than from any inherent defect in the natural drainage. Tick-infested grazings in the remainder of the zone are restricted to rush-ridden *Agrostis* pastures or to fescue-*Agrostis* pastures, often invaded by bracken-fern, at higher elevations.

The extent of infested grazings in the zone can be inferred from fig. 2 which shows a transect made in the Dolybont district. The area depicted is about 475 acres and its elevation lies between 50 ft. and 350 ft. The fields marked (C) include not only those under the plough in 1948 but all new seeds put down during 1946 and 1947. *Agrostis* pastures form 24.5 per cent. of the total, *Agrostis* with rye-grass pastures 9.0, rush-ridden fields 9.6 and woodland 6.1 per cent. Tick-infested pastures covered only 9.0 per cent. of the total area and were situated on poorly drained land near the river Leri. These fields were susceptible to periodic flooding. Not all rough grazings were tick-infested and on two steadings, pastures similar in composition to the tick-infested grazings in the river valley were tick-free.

The tick-infestation of cattle on two farms in the transect was noted at seven- to 12-day intervals between April and July 1947. The infestations recorded on the two steadings were significantly different and respectively characterised the heavy (over 100 ticks per cow) and the light (below 50 ticks per cow) infestations apparent on cattle in the zone. Farm A is a small holding comprising 10 acres, 7 acres of which are tick-infested. The farmstock consisting of three dairy cows are confined to the infested pastures from March to December except for intermittent periods when they have access to the tick-free *Agrostis* with rye-grass pasture. The continuous stocking of the poorer grazing has resulted in a build-up of a heavy tick-infestation of the pastures. A peak infestation of 301.0 ticks per cow was recorded in 1947. On farm B, however, only 13 of the 40 acres of available grazing land are tick-infested. According to the prevailing husbandry methods, store-cattle graze the infested

pastures at intervals from March to October while the dairy cattle are occasionally turned out to these fields between April and October. The heaviest infestation recorded on the 14 store-cattle and the 12 dairy cows was respectively 37.4 and 8.7 female ticks per beast. The short periods for which cattle graze the infested pastures on this farm limits the number of ticks that can complete their life-cycle. This directly controls the rate of increase of the tick population and, in this instance, results in the infestation of the pastures remaining at a low level.

Within the *Agrostis* zone, the majority of the tick-infested steadings resemble farm B in the extent of infested grazings and in the degree of infestation of the farmstock. This is evident in Table I where the estimated peak infestation of cattle on 56 steadings, out of a total of 66 tick-infested, was below 50 ticks per cow.

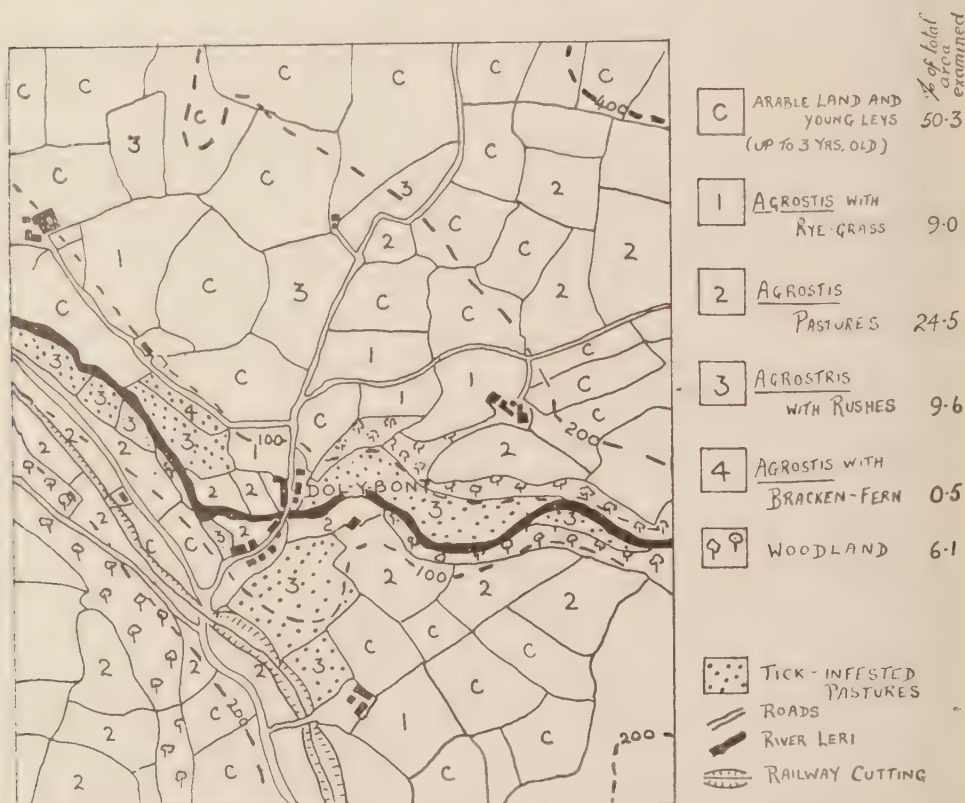


Fig. 2.—The extent of tick-infested pastures in a lowland district in N. Cardiganshire.

(b) Lowland Bog zone.

This zone is formed by the Borth Bog and covers over 5,000 acres of land in the north-western portion of fig. 1. A large tract of this marshland is excessively wet and of no agricultural value. Much of it lies at or near sea-level and is therefore affected by salt water. Rushes, heather (*Calluna vulgaris*), deergrass (*Scirpus caespitosus*), cotton grass (*Eriophorum* spp.), mat grass (*Nardus stricta*) and flying bent (*Molinia caerulea*) all contribute to the herbage of the Bog. The steadings are situated around its margin, often on poorly drained land. On a number of the farms large tracts of the marshland have been drained, ploughed and reseeded.

Cattle were examined on 19 farms and tick infestations of a varying intensity were recorded on 16. The tick-infested grazings covered extensive areas and were predominantly *Molinia-Nardus* pastures with rush and cotton grass. The improved pastures on some steadings were either tick-free or supported a low infestation. This resulted, as in the *Agrostis* zone, in the occurrence of two distinct types of tick-infested steadings differing in the degree of infestation of the farmstock according to the acreage of tick-free grazings on the farm. The heavy infestations of cattle were confined to farms on which the infested pastures formed a high percentage of the available grazing land.

(c) *Fescue-Agrostis* zone.

Fescue-Agrostis pastures predominate at elevations between 600 and 1,000 ft. and even though the swards are densely matted there is seldom any peat. Over wide areas these pastures have been colonised by bracken-fern and gorse. The zone is not, at present, intensely cultivated. During the last 30 years a large number of the farms has been vacated and the land grouped into larger steadings resulting in the one-time mixed farming being replaced by a ranching system with sheep. In the course of the present survey cattle and sheep were examined on 17 steadings. All proved to be heavily tick-infested.

A transect made in the Glandyfi district is shown in fig. 3. The map represents approximately 580 acres of land lying between 400 and 1,000 ft. The distribution of grasslands shows a dominance of *fescue-Agrostis* pastures comprising 39.6 per cent. of the total area. These neglected pastures were in all cases invaded by bracken-fern or gorse. At higher elevations, above 800 ft., mountain *fescue* with *Nardus* was dominant and with *fescue-Agrostis* pastures form the hill grazings for sheep and store cattle. Arable land (C) and *Agrostis* pastures, forming 13.6 per cent. of the area, are confined to fields near the farmstead. Tick-infested grazings are extensive and cover 81.0 per cent. of the total area. Sheep are restricted to the hill grazings except for lambing when the majority of the ewes are brought down to the sheltered fields around Cwmere. The average peak infestation of five sheep and five dairy cattle on this farm during the spring of 1948 was 32.6 ticks per sheep and 79.2 ticks per cow. In autumn the cattle recorded a peak infestation of 222.0 ticks per beast.

The tick infestation of farmstock on other holdings in the zone was generally of the same intensity although, on two farms, cattle grazing on well-managed pastures around the farmsteads recorded lower infestations than the sheep grazing hill pastures.

(d) *Molinia-Nardus* zone.

This zone is essentially one of hill grazings at elevations over 1,000 ft. In this region it is dominated by rough grazings consisting of *Molinia-Nardus* moorland with restricted areas of *fescue-Agrostis* pastures with bracken-fern and rush. In favourable spots hill farms have been established and around the farmsteads small areas of cultivated land. A large number of the farms now lie derelict and the land forms mountain grazings for the sheep of marginal and lowland farmers.

Tick-infested pastures form extensive areas and generally cover the whole of the grazing land of a steading. Sheep examined from 17 farms were all heavily infested and store-cattle, when present, gave infestations of between 100 and 300 ticks per beast.

(iv) *Economic importance.*

I. ricinus is responsible for two important diseases of farmstock in N.W. Cardiganshire, bovine piroplasmiasis and tick pyaemia of lambs. Besides losses due to these specific diseases various harmful effects can result from heavy tick

Examined
area
% of total

1	ARABLE LAND AND LEYS (UP TO 3 YRS. OLD)	6.7
2	AGROSTIS PASTURES	3.8
3	AGROSTIS WITH RUSHES	3.1
4	FESCUE - AGROSTIS WITH BRACKEN-FERN	35.1
5	FESCUE - AGROSTIS WITH GORSE	4.1
6	MOUNTAIN FESCUE WITH NARDUS	38.6
7	MOLINIA AND NARDUS	2.2
8	WOODLAND	6.2
	TICK-INFESTED PASTURES	
	RIVER BRWYNO	

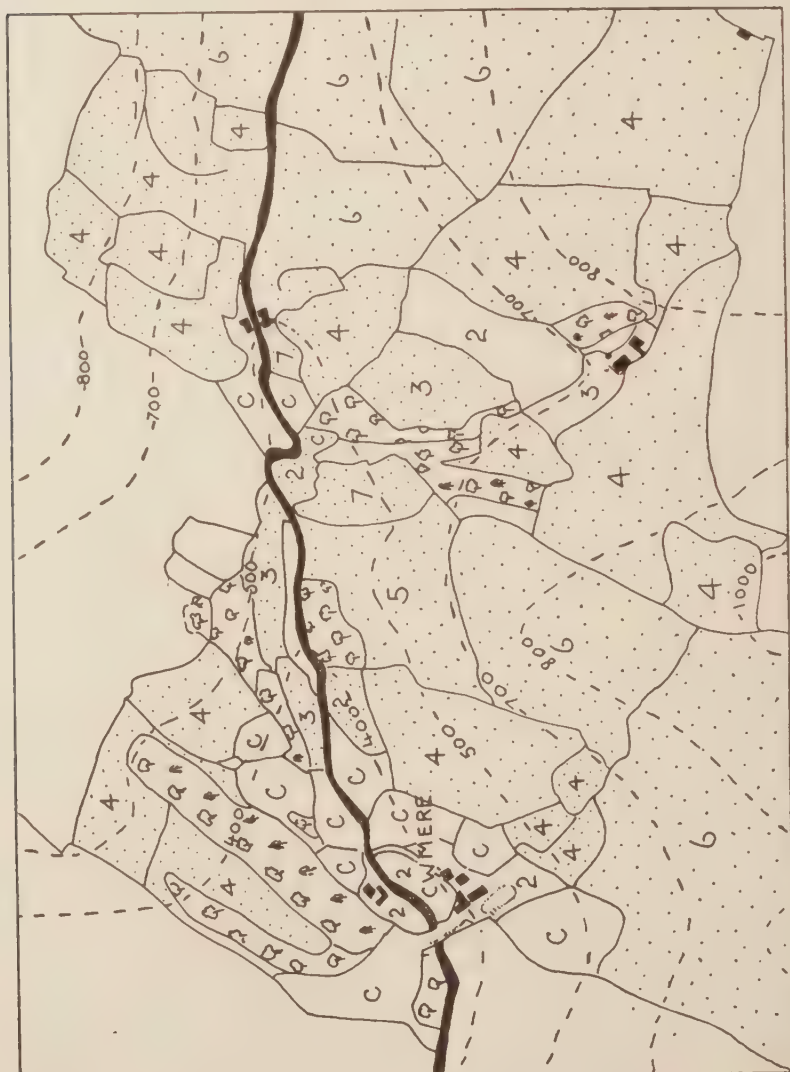


Fig. 3.—The distribution of tick-infested pastures on Cwmere farm, Glandyfi, N. Cardiganshire.

infestations. Infested sheep rub and scratch in an attempt to rid themselves of the irritation caused by the tick and thereby depreciate the wool clip. A heavy tick-infestation of cattle causes "tick worry" characterised by restlessness and interference with grazing. The loss of blood from a heavy and prolonged infestation must be appreciable and many attempts have been made to estimate it. According to MacLeod (1939), the blood ingested by ticks represents only a part of the total blood loss since the biting tick injects an anticoagulant into the wound and, as it detaches, a clot is slowly formed over the puncture. In a larval infestation the blood loss is greater after the tick detaches than the amount ingested. In Britain anaemia is, nevertheless, a rare sequel to tick infestation.

In this survey particular attention has been given to the incidence of bovine piroplasmosis and tick pyaemia.

(a) Bovine piroplasmosis.

The occurrence of this disease on farms situated in the major grassland zones of the region is given in Table II.

TABLE II.

The distribution of bovine piroplasmosis on farms in N.W. Cardiganshire in relation to the major zones of grassland.

Zone	No. of tick-infested farms	No. of tick-infested farms reporting piroplasmosis	Percentage tick-infested farms endemic for piroplasmosis
<i>Agrostis</i> ...	66	40	60.6
Lowland Bog. ...	17	9	47.4
Fescue- <i>Agrostis</i> ...	17	3	17.6
<i>Molinia-Nardus</i> ...	10*	?	?

*Cattle present on three farms only.

Farms endemic for piroplasmosis form over 50 per cent. of the tick-infested steadings in this region. Approximately 94 per cent. of the infested farms occur in the *Agrostis* and the Lowland Bog zones while in the two zones at higher elevations there were only three holdings reporting recent outbreaks of the disease. This variation between the zones is primarily due to the distribution of cattle within the region. In the lowland zones, where approximately 70 per cent. of the farms are situated, mixed farming predominates although in recent years there has been an increase in dairy farming. In the fescue-*Agrostis* and the *Molinia-Nardus* zones sheep farming predominates and cattle, when present, are kept as stores; the isolation of the steadings making milk production impracticable. The type of farming in the lowland zones results in a continuous movement of cattle, immune or non-immune to piroplasmosis, between the farms. Non-immune cattle brought on to farms endemic for piroplasmosis invariably contract the disease whereas the introduction of infected cattle on to tick-infested steadings, free from the disease, may initiate piroplasmosis on the farm. The rearing of store cattle on infected farms in the fescue-*Agrostis* and the *Molinia-Nardus* zones results in the young stock acquiring an immunity to the disease at an early age and it is only when non-immune cattle are exposed to infection that outbreaks of piroplasmosis occur. This phenomenon is evident at Cyneiniog farm in the region. The farm was vacated 23 years ago and subsequently the land became hill grazings for sheep and store cattle of a lowland farm. During the period preceding its vacation piroplasmosis was of rare occurrence. The recent stocking of the tick-infested pastures with non-immune

cattle from the lowland farm has caused an annual outbreak of the disease and the farmer was forced to return his cattle to lowland grazings in 1948 after losing four of them.

The seasonal incidence of piroplasmosis in the region during 1944, 1945 and 1946 is given in Table III.

TABLE III.

The seasonal incidence of bovine piroplasmosis in N.W. Cardiganshire during 1944, 1945 and 1946.

Year	Number of cases of bovine piroplasmosis treated by veterinary practitioners during the following months												Total No. of cases treated
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	
1944 ...	0	0	0	0	13	6	8	13	14	20	11	0	85
1945 ...	0	0	0	0	18	17	15	26	28	27	3	3	137
1946 ...	0	0	0	3	8	21	45	39	20	11	7	1	155
Total ...	0	0	0	3	39	44	68	78	62	58	21	4	377

Between January 1944 and December 1946, 377 cases of bovine piroplasmosis were treated by veterinary practitioners. The occurrence of the disease coincides with the period of tick activity, *i.e.*, from March to December (Evans, 1950) but the number of cases treated monthly is not an index of the degree of tick activity. Factors such as delayed stocking of infested pastures, the period at which non-immune cattle are taken on infested farms, and the time lag between infection and the development of clinical symptoms of the disease may extend the period of treatment beyond the spring and autumn phases of tick activity.

(b) Tick pyaemia.

This disease is confined to lambs on hill grazings in this region. The mortality rate is not high and at its heaviest seldom exceeds 5 per cent. of the lamb crop. Lambs which recover are always unthrifty with the result that the farmer has fewer saleable lambs than if his flock was free from the disease. In recent years lambs on a number of lowland farms have been dipped in an anti-tick preparation before being taken to hill grazings in May. According to the farmers this treatment has caused a substantial decrease in the incidence of pyaemia in their flocks. Similar treatment does not appear to be practicable on hill farms where losses continue to occur under the prevailing system of sheep management.

(v) Conclusions.

The widespread distribution of *I. ricinus* in N.W. Cardiganshire shows a significant degree of correlation with areas of rough grazing. The tick-infested pastures invariably contained one of the following plants as a dominant constituent of the vegetation: fine-leaved fescue (*Festuca rubra* and *F. ovina*), bent grass (*Agrostis* spp.), mat grass, flying bent, bracken-fern and rush. Rough grazings predominate in the fescue-*Agrostis* and the *Molinia-Nardus* zones at higher elevations, where the soils and exposure provide naturally poor grasslands. In the *Agrostis* zone tick-infested pastures are restricted to isolated tracts of poorly drained or badly managed land where rush and bracken-fern has colonised the pastures. Fescue-*Agrostis* pastures, invaded by bracken-fern, at higher elevations also provide suitable areas for tick-infestation. The Borth Bog region, resembling the *Molinia-Nardus* zone in vegetation, forms an extensive area of low-lying tick-infested grazings.

The degree of tick infestation of the cattle of the steadings in the region depend on the percentage of tick-infested pasture to the total available grazing land. A small proportion of infested pasture to the total grazing land results in a light infestation of the stock whilst cattle practically confined to infested grazings record heavy infestations irrespective of the position of the steading in relation to the grassland zones.

Economically, bovine piroplasmosis is the most important tick-borne disease of the region. Its distribution is practically confined to the infested pastures of the lowland zones. Outbreaks of the disease in the fescue-*Agrostis* and *Molinia-Nardus* zones are infrequent and occur when non-immune cattle are brought on to the farms. Tick pyaemia is confined to hill grazings and the incidence of the disease is not high.

The general Distribution and economic Importance of the Tick in Wales and the Welsh Border Counties.

The distribution of the tick in Wales and the border counties of Hereford and Salop was recorded by means of a questionnaire despatched to farmers and by the examination of cattle and sheep in widely separated areas of the country. In addition to questions on the tick, the questionnaire included a section on the sheep ked *Melophagus ovinus* (L.). It was intended in this way to assist the farmer in differentiating between the two ectoparasites, a factor of great importance as these pests are often collectively called the "sheep tick". The data received concerning the habits of the ked also provided a useful supplement to the investigations already carried out on this insect by the writer (Evans, 1946, 1950). The language difficulty was overcome by despatching English and Welsh copies of the questionnaire to each farmer.

The incidence of bovine piroplasmosis is based on the replies received. Veterinary practitioners in selected counties also supplied details of the number of cases of piroplasmosis treated by them during 1945 and 1946. An estimate of the mortality rate due to the disease in Cardiganshire and Carmarthenshire was obtained from the analysis of records kept by knackerery owners.

General distribution.

During the course of this survey, 2,050 questionnaires were despatched to farmers in Wales and the Welsh border counties and 385 were returned (18·8 per cent.). An analysis of the data is given in Table IV and the localities from which the tick was reported are shown in fig. 4.

It is evident that the tick predominates in the western counties of the region studied. This distribution is closely connected with the occurrence of rough grazings in the counties. With the exception of the counties of Carmarthen, Pembroke, Radnor and Brecon there is a marked relationship between the incidence of the tick and the percentage of agricultural land under rough grazing (Table IV). The extent of tick-infested grazings within a county is largely dependent on the area of land suitable for intensive farming. In Merionethshire the agricultural land forms a mere fringe between moorland and sea. Tick-infested pastures are extensive and cover the major part of the marginal and hill grazings. The lowland belt in Carmarthenshire and Pembrokeshire on the other hand, forms the major area of the counties with the result that there are few widespread areas of infested grazings. The tick-infested pastures in these counties are in the main confined to small tracts of poorly drained and badly managed grazings in the *Agrostis* zone. This accounts for the relatively small area under rough grazings as opposed to the high incidence of tick-infested steadings.

TABLE IV.
The incidence of *Ixodes ricinus* in Wales and the Welsh border counties based on replies received from questionnaires despatched during 1947.

County	No. of questionnaires despatched	No. of replies received	Percentage of replies received	No. of farms reporting tick on			Percentage of farms tick infested	Percentage of total agricultural land under rough grazing *
				Cattle only	Sheep only	Cattle and Sheep		
Anglesey ...	54	8	14.9	2	1	2	62.5	13.7
Brecon ...	196	36	18.4	0	6	2	22.2	63.2
Caernarvon...	200	57	28.5	3	4	39	87.0	52.6
Cardigan ...	150	27	18.0	4	3	9	59.3	41.4
Cardarthen ...	103	18	17.5	3	0	8	20.2	20.2
Denbigh ...	147	13	8.8	0	3	1	30.8	36.6
Flint ...	94	14	14.9	0	0	1	7.1	9.5
Glamorgan ...	156	26	16.7	4	5	3	46.2	39.2
Merioneth ...	145	43	29.7	4	5	26	81.0	63.0
Monmouth ...	100	5	5.0	0	0	0	0.0	20.3
Montgomery ...	150	28	18.7	1	2	13	57.1	42.5
Pembroke ...	103	22	19.8	6	1	6	59.1	18.6
Radnor ...	25	25	24.8	1	5	1	28.0	45.6
Hereford ...	198	33	16.7	0	2	0	6.1	6.5
Salop ...	152	30	19.7	1	0	1	6.7	7.3
Total ...	2,050	385	18.8	29	37	112	47.5	31.8

*Based on Agricultural Statistics (1935).

Breconshire and Radnorshire, counties with extensive areas of rough grazings, have a surprisingly low incidence of tick. The small number of infested steadings reported in the questionnaires has been supported by veterinary practitioners in the counties. The low incidence may be due to the recent establishment of the tick in the counties since agriculturally they are within easier reach of the lightly-infested eastern counties than they are with the heavily infested counties of the west.

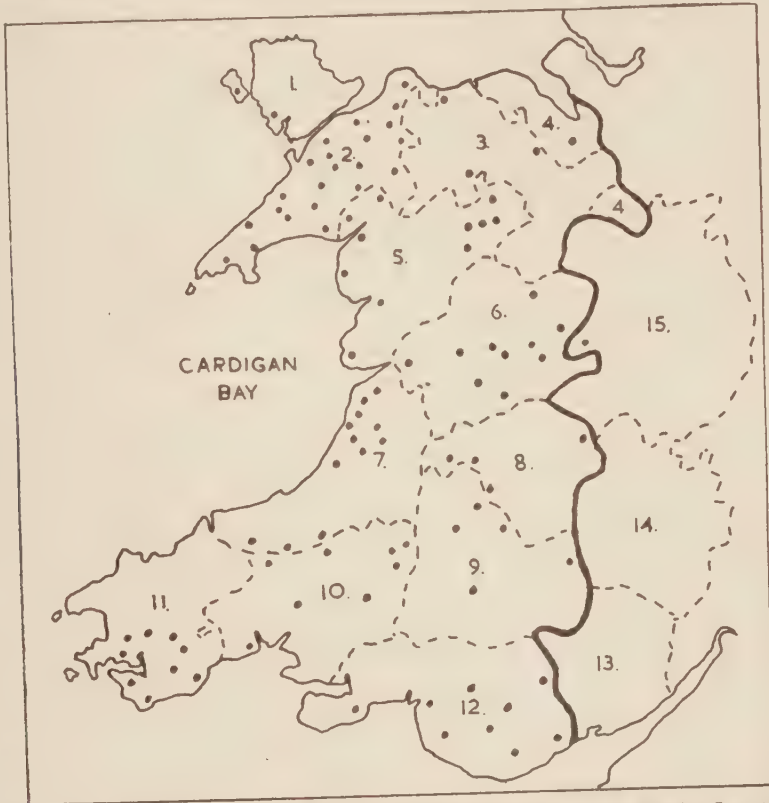


Fig. 4.—The distribution of *Ixodes ricinus* in Wales and the Welsh Border Counties.

- | | | |
|---------------------|----------------------|-------------------------|
| 1. Anglesey. | 6. Montgomeryshire. | 11. Pembrokeshire. |
| 2. Caernarvonshire. | 7. Cardiganshire. | 12. Glamorganshire. |
| 3. Denbighshire. | 8. Radnorshire. | 13. Monmouthshire. |
| 4. Flintshire. | 9. Breconshire. | 14. Herefordshire. |
| 5. Merionethshire. | 10. Carmarthenshire. | 15. Shropshire (Salop). |

Economic importance.

The incidence of bovine piroplasmiasis in Wales and the Welsh border counties as reported in the questionnaires is given in Table V.

The disease is prevalent in the heavily tick-infested counties of the west and appears to be absent in the border counties. Merionethshire and Montgomeryshire have a relatively low incidence in relation to the widespread distribution of the tick. In the former this may be due to the tick predominating on the marginal and hill farms which for reasons given above (p. 477) have infrequent outbreaks of the disease. It is probable that in Montgomeryshire the low incidence is a reflection of the small number of farms which are endemic for piroplasmiasis. It is interesting to note the

TABLE V.

The incidence of bovine piroplasmosis in Wales and the Welsh-border counties as abstracted from questionnaires despatched during 1947.

County	No. replies received to questionnaire	No. farms reporting tick on cattle	No. farms reporting piroplasmosis	Percentage of farms with tick infested cattle	Percentage of farms reporting piroplasmosis
Anglesey ...	8	4	1	50.0	12.5
Brecon ...	36	2	1	5.5	2.8
Caernarvon ...	57	42	25	73.7	43.9
Cardigan ...	27	13	8	48.1	29.6
Carmarthen ...	18	11	8	61.1	44.4
Denbigh ...	13	1	1	7.6	7.6
Flint ...	14	1	0	7.1	0.0
Glamorgan ...	26	7	3	26.9	11.5
Merioneth ...	43	30	11	70.0	25.6
Monmouth ...	5	0	0	0.0	0.0
Montgomery ...	28	14	2	50.0	7.1
Pembroke ...	22	12	4	54.5	18.2
Radnor ...	25	2	0	8.0	0.0
Hereford ...	33	0	0	0.0	0.0
Salop ...	30	2	0	6.7	0.0

widespread occurrence of the disease in Carmarthenshire where tick-infested grazings predominate on farms in the lowland regions.

The number of cases of piroplasmosis treated by veterinary practitioners in selected regions of Wales (Table VI) support the general picture obtained by the questionnaire method. The two cases reported in Radnorshire on November 1946 were in animals recently imported from Carmarthenshire.

The mortality rate among cattle in Cardiganshire and Carmarthenshire from July 1947 to June 1948 shows the importance of this disease in the two counties (Table VII). The 110 animals collected by knackery owners does not take into account the number of dead animals disposed of by other means, e.g., burial on the farm, so that the actual mortality rate may be much higher than that recorded.

Conclusions.

The dominant feature controlling tick distribution appears to be the distribution of rough grazing. The western counties of Wales are the major tick areas whilst the border counties are practically free from the parasite.

Bovine piroplasmosis is widespread in the heavily tick-infested counties. The lowland farms report a higher incidence of the disease than the marginal and hill farms.

Discussion.

The survey of the distribution of *I. ricinus* in N.W. Cardiganshire demonstrated the economic importance of small tracts of infested grazings at elevations below 600 ft. In N.W. Northumberland, Milne (1944) found that farmlands below 1,000 ft. were almost entirely free of the tick, infested areas being confined to hill grazings between 1,000 and 2,000 ft. In Northumberland the elevated regions are more extensively farmed than in Cardiganshire with the result that there is not a comparable movement of farmstock between lowland and hill farms. This together with fewer pastures suitable for tick colonisation in the lowland regions of

TABLE VII.

The mortality rate among cattle in Cardiganshire and Carmarthenshire as the result of piroplasmosis.

County	No. of cattle which died of piroplasmosis and taken by knackeries during the following months												Total
	1947						1948						
	J.	A.	S.	O.	N.	D.	J.	F.	M.	A.	M.	J.	
Cardigan	5	1	8	5	2	0	0	0	0	0	9	16	46
Carmarthen	9	3	11	8	2	0	0	0	0	4	13	14	64

Northumberland may account for the marked difference in the distribution of the tick in the two regions.

The importance of lowland infested pastures in Wales suggests the possibility of controlling the tick by pasture improvement. The majority of these pastures could be improved by simple drainage and reseeding. The removal of the matted vegetation not only results in an immediate decrease in the tick population but under good management may effect its eradication. In recent years this procedure has been successful in the Borth Bog region of N. Cardiganshire. On the marginal and hill farms the problem of pasture improvement is far more difficult. Tick-infested grazings form extensive areas on inherently poor land which has deteriorated with the vacation of large numbers of the holdings. In these areas the farmer is forced to treat his stock with anti-tick preparations to decrease the infestation. This method is extremely laborious and on several farms no control of the tick is attempted.

The high incidence of bovine piroplasmosis in the heavier tick-infested counties of Wales is an economic problem of major importance. Its widespread occurrence on lowland farms warrants the eradication of the tick from these regions. In addition, the intake of infected stock from Carmarthenshire and Montgomeryshire into the counties of Radnor and Brecon may have serious repercussions in these counties as they have a low incidence of the disease but areas suitable for tick infestation.

Summary.

Ixodes ricinus is the common tick infesting farmstock in Wales. *Dermacentor reticulatus* is recorded on cattle and sheep on two farms in N. Cardiganshire.

I. ricinus is widely distributed in the four major grassland zones of N.W. Cardiganshire. The most extensive areas of tick infestation are found on the marginal and hill farms. In lowland areas, except on extensive stretches of marshland, infested pastures are confined to isolated tracts of badly managed or poorly drained land. Infested grazings are invariably "rough" and contain one of the following plants as dominant: *Festuca* spp., *Agrostis* spp., *Molinia caerulea*, *Nardus stricta*, *Pteris aquilina* or *Juncus* spp. The degree of infestation of cattle depends on the relative area of the available grazing land colonised by the tick. Continuous stocking of infested pastures results in heavy infestations and *vice versa*.

Bovine piroplasmosis is the major tick-borne disease in N.W. Cardiganshire and predominates in the lowland districts. Outbreaks of the disease on marginal and hill farms are generally infrequent. Reasons are suggested for this phenomenon. Tick pyaemia is confined to lambs on hill grazings. The incidence of this disease is low.

The distribution of the tick in Wales and the border counties of Hereford and Salop shows a general relationship to areas of rough grazing. The tick is widespread in the western counties and is almost absent in the border counties. Radnorshire and Breconshire are interesting in that they have few infested farms in relation to the extensive nature of the "rough" grazing. A survey has also been conducted of the incidence of bovine piroplasmiasis in Wales.

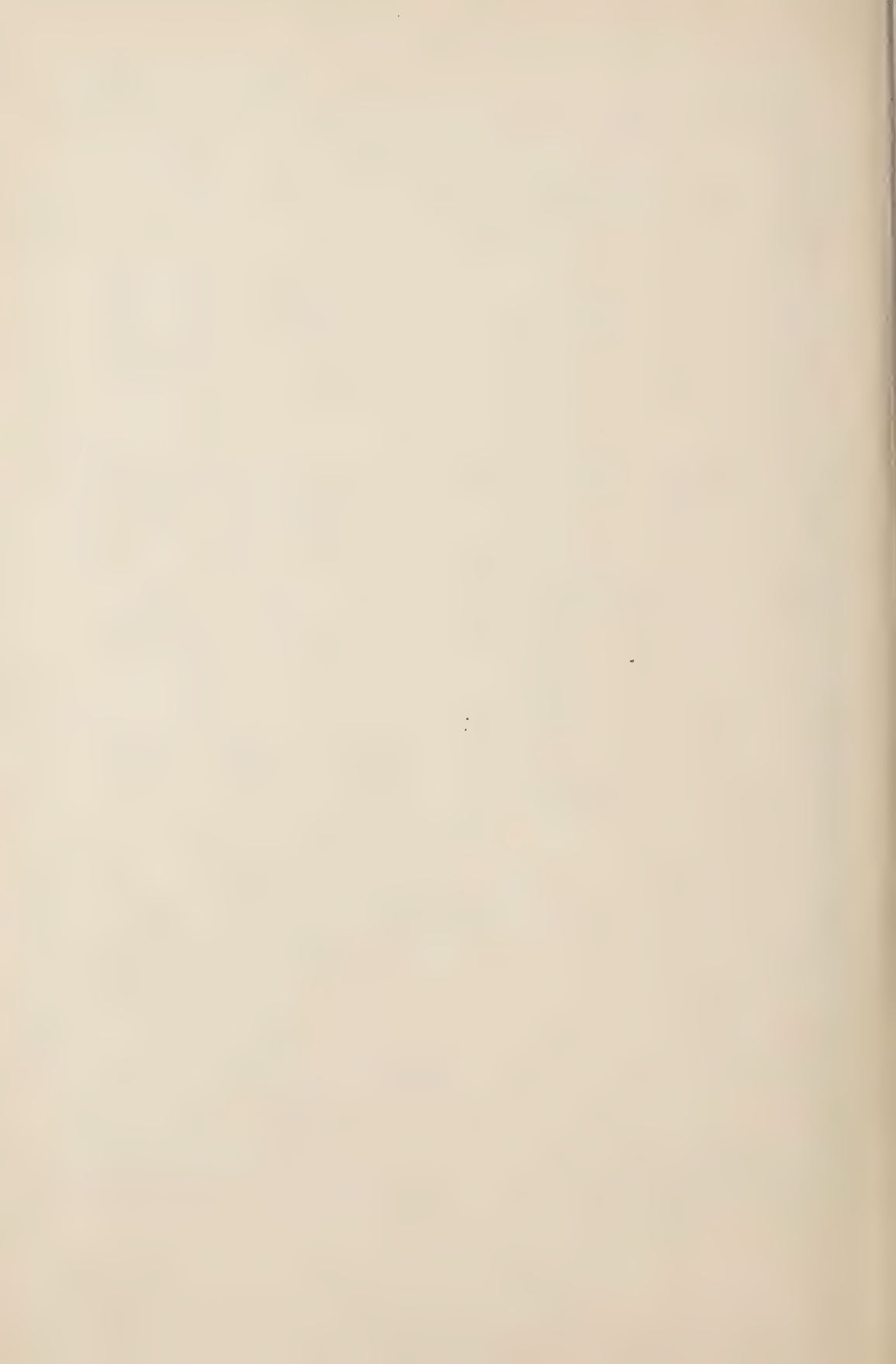
Acknowledgements.

I am indebted to Cooper and MacDougall Ltd. for the grant with which this work was undertaken. Also to Prof. A. N. Worden, Aberystwyth, and Mr. W. Downing, Cooper Technical Bureau, for their interest and advice.

I wish to acknowledge the assistance received from veterinary surgeons and farmers in various districts visited during the course of the survey.

References.

- CAMPBELL, J. A. (1946). Scot. Fmr, **54**, p. 1331.
- DAVIES, W. (1936). The grasslands of Wales—a survey ~~by~~ ^{by} Stapledon, R. G. A survey of the agricultural and wastelands of Wales, pp. 13–107. London, Faber & Faber. h
- EDWARDS, E. E. & ARTHUR, D. R. (1947). Parasitology, **38**, pp. 72–85.
- EVANS, G. O. (1946). Nature, Lond., **157**, p. 773.
- EVANS, G. O. (1950). Bull. ent. Res., **40**, pp. 459–478.
- JONES, O. T. (1911). Nat. Un. Teach. Souvenir of Aberystwyth Conference, 1911, pp. 25–51.
- MACLEOD, J. (1932). Parasitology, **24**, pp. 382–400.
- MACLEOD, J. (1935). *Ibid.*, **27**, pp. 123–144.
- MACLEOD, J. (1939). Emp. J. exp. Agric., **7**, pp. 97–110.
- MACLEOD, J. & GORDON, W. S. (1932). J. comp. Path., **45**, pp. 240–256.
- MACLEOD, J. & GORDON, W. S. (1933). Parasitology, **25**, pp. 275–283.
- MILNE, A. (1944). *Ibid.*, **35**, pp. 186–196.
- NAT. VET. MED. ASS. GT. BRIT. (1945). Parasitic diseases of cattle.—Publ. nat. vet. med. Ass. Gt. Brit., no. 8.
- STEWART, W. L. (1941). J. R. agric. Soc. Engl., **101**, pt. 2, pp. 57–62.
- WALTON, C. L. (1927). Parasitology, **19**, pp. 265–273.
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STUDIES ON SALT-WATER AND FRESH-WATER *ANOPHELES GAMBIAE* ON THE EAST AFRICAN COAST.

By R. C. MUIRHEAD THOMSON, D.Sc.

Colonial Medical Research.

(Plate XI.)

In the last few years a considerable amount of work has been carried out in West Africa on the relationship between *Anopheles gambiae* Giles and what used to be called *A. gambiae* var. *melas* Theo. This latter name was given to mosquitos which differed from typical *gambiae* mainly in that the females had an additional dark band on the palps, and which were associated with brackish water and coastal regions. The facts now at our disposal indicate that we are really dealing with a distinct species, *A. melas*, which differs from typical *gambiae* not only by its different breeding habits, but also on morphological characters of the egg (Muirhead Thomson, 1945) and larva (Ribbands, 1944), and the physiological reactions of the larvae (Ribbands, 1944). Further evidence of the distinct nature of the species was provided by cross-fertilisation experiments (Muirhead Thomson, 1948). Records of mosquito dissections based on exact identification of the adult have also shown that *A. melas* is an efficient vector of malaria (Tredre, 1946; Muirhead Thomson, 1948).

Whilst the situation in West Africa is now much clearer, information from East Africa has lagged behind. The occurrence of *A. gambiae* breeding in brackish water in East Africa has been recorded from time to time, and where melanic adults with an additional dark band on the palps have been found, they have been referred to as "*A. gambiae* var. *melas*" (Mackay, 1938; Wilson, 1936).

On the other hand salt-water breeding of *A. gambiae* has long been recognised in Mauritius, but "var. *melas*" adults have not been recorded (Jepson & others, 1947).

Mackay (1938) failed to find any "var. *melas*" infected in Dar-es-Salaam but the number of specimens dissected was not recorded.

The limits of East African "var. *melas*" have obviously not been clearly defined, and its rôle in malaria transmission is still uncertain.

Identity of salt-water *A. gambiae*.

Dar-es-Salaam, Tanganyika, was selected as the most suitable spot in which to study salt-water breeding of *A. gambiae* and shortly after the writer's arrival in July 1947 a focus of salt-water breeding was found near the coast road, 4 miles north of Dar-es-Salaam. The little village of Changombe at this point was the main centre of field investigations for the following 14 months.

Periodical breeding of *A. gambiae* was found to occur in swampy patches of *Paspalum* grass along the extreme upper tidal limit, only reached by the highest spring tides. A certain amount of breeding was also found in open spaces among small *Avicennia* mangrove bushes, under conditions resembling those in West Africa. But the densest and most continuous breeding was found in shallow, bare-edged, brackish water ponds up to half a mile inland from the tidal belt, and having no connection with the sea (Pl. XI, fig. 1). At first these ponds were taken to be typical peaty pools, with dark water and scarcity of vegetation, but tests showed a salinity of roughly 50 per cent. sea water. The larvae taken from them showed no distinct morphological differences from those of typical *gambiae*, and this applied particularly

to the characters of the larval pecten, which form a fairly reliable method of distinguishing *gambiae* from *melas* in West Africa. A variable proportion, up to about 50 per cent., of the adults bred from these brackish ponds had an additional dark band on the female palps—resembling four-banded *melas* from West Africa—the remainder being indistinguishable from typical *gambiae*. Finally the egg characters were investigated, and it was found that eggs collected from brackish ponds, as well as those laid by all "*gambiae*" females caught in houses beside the breeding place, were indistinguishable from those of typical *gambiae*.

The evidence, then, indicates that the salt-water form of *A. gambiae* on the East African coast is not the same as *A. melas* in West Africa. The differences discovered so far may be summarised as follows:—

		West African <i>A. melas</i>	East African salt-water <i>A. gambiae</i>
Larva	...	Can usually be distinguished from those of typical <i>gambiae</i> by characters of pecten.	Indistinguishable from those of typical <i>gambiae</i> .
Adult...	...	Variable proportion of females with additional dark band on palps, <i>i.e.</i> , 4-banded forms.	As in <i>A. melas</i> .
Egg	...	Distinctly different from that of typical <i>gambiae</i> .	Similar to that of typical <i>gambiae</i> .

In West Africa the clear-cut difference between the eggs of *gambiae* and *melas* enabled adult female mosquitos caught in houses to be identified with certainty by the eggs they laid in captivity. This fortunate state of affairs evidently does not apply in East Africa, and the egg characters are useless for identifying wild-caught females.

It is possible that a detailed and prolonged study of these larvae and adults might reveal some constant morphological difference, but in the present investigation it was necessary to find some simple way of distinguishing adults of the two forms as early as possible, so that their incidence in houses, distribution, and rôle in malaria transmission could be compared. A physiological difference was, therefore, resorted to.

In West Africa it was known that when larvae were put into 37.5 per cent. sea water (—11.9 gm. NaCl per litre) for 24 hours and then transferred to 75 per cent. sea water (23.8 gm. NaCl per litre) for a further 24 hours, all the larvae of the salt-water breeder, *melas*, survived, while those of typical fresh-water *gambiae* succumbed (Ribbands, 1944). In East Africa this test was modified to enable adults to be identified quickly.

Adult female "*gambiae*" caught in village houses in coastal areas, were isolated in small 6 × 6-inch cages containing a bowl or saucer of water, with a few blades of grass floating on the surface to facilitate oviposition. Eggs were usually laid within two or three days, and were then transferred by means of a wire loop to a beaker of water. Floating on the surface of the beaker was a flat paper ring on which was written the index number and date corresponding to that of the cage of origin. The eggs were transferred to the water within this paper ring, so that there was no chance of their being stranded on the glass sides of the beaker. When the larvae hatched, usually in another two days, they were transferred directly to 75 per cent. sea water (a solution of 23.8 gm. NaCl per litre). The young larvae of fresh-water *gambiae* from inland areas were all dead within two hours, while those of salt-water *gambiae* survived at least six hours.

Although this is rather a lengthy procedure, the difference in the reaction is very distinct, and has formed the basis of all work on the identification of adults.

The Incidence of salt- and fresh-water *A. gambiae* Adults in native Houses round Dar-es-Salaam.

Salt-water *gambiae* were found breeding continuously in August, September and October 1947 in the brackish swamps and ponds round Changombe, but at no other place near Dar-es-Salaam. In order to find out if there were any undetected breeding grounds in other localities, and also to ascertain to what extent salt-water *gambiae* extended inland, collections of mosquitos were made in African houses in four localities (fig. 1). The identity of all "*gambiae*" caught was determined by salt tests on their progeny as described above.

The results are shown in Table I.

TABLE I.

Incidence of fresh-water and salt-water *A. gambiae* in African houses in 4 localities round Dar-es-Salaam, August to October, 1947.

	Total adults caught		" <i>gambiae</i> " tested	Salt-water forms	Fresh-water forms
	<i>funestus</i>	" <i>gambiae</i> "			
Changombe (16 collections)	103	559	203	117	86
Kinondoni (7 collections)	127	157	52	4	48
Kigogo (4 collections)	294	168	88	1	87
Kivukoni (3 collections)	136	16	9	0	9

These figures show that at this period of the year, when salt-water breeding is at its height, there was very little indication of salt-water adults far from Changombe. Kigogo, only a mile inland from the mangrove belt of Msimbazi Creek, is almost a pure fresh-water *gambiae* area. Kivukoni, south of the harbour, is mainly a *funestus* Giles area, and the few *gambiae* caught there proved to be fresh-water forms.

The catches at Kinondoni are particularly interesting. These houses are separated from the township by the tidal part of the Msimbazi Creek, most of which is occupied by an extensive *Avicennia* orchard. In West Africa such an orchard would have formed an ideal breeding ground for *melas*, but larvae were never found in this East African orchard. The scarcity of salt-water breeding in the creek is indicated also by the low proportion of salt-water *gambiae* adults in the houses.

It is only a few years since Msimbazi Creek was a notorious breeding place of salt-water *gambiae*, and there is little doubt that the extensive drainage schemes have eliminated most of the brackish swampy ground which used to exist. Other reasons for the scarcity of salt-water *gambiae* here will be discussed later.

The house catches at Changombe show that salt- and fresh-water *gambiae* are usually taken together in varying proportions.

The seasonal incidence of the Anopheline vectors—*gambiae* and *funestus*—in houses in the Dar-es-Salaam area has been reported on by Wilson (1946) for the years 1943–44–45, and those figures show an annual peak of vectors from April to

July. The peak starts with a great production of *gambiae* at the beginning of the rainy season, but later on *funestus* appears in increasing numbers. Conditions vary much from year to year, and even in the present investigation 1947 was very different from 1948.

It was originally intended to work out the relative proportion and abundance of the two forms of *gambiae* in houses, in the same systematic way as was done for *gambiae* and *melas* in Lagos. For various reasons this widespread sampling was not possible in Dar-es-Salaam, and attention was concentrated on the village of Changombe. The proportion of the two forms in the house catch has been worked out, but as the catches were not standardised the totals give only an approximate idea of the relative abundance from month to month.

The results are shown in Table II.

TABLE II.

Catches (unstandardised) of salt-water and fresh-water *A. gambiae* in houses at Changombe (Dar-es-Salaam) 1947-48.

			Female " <i>gambiae</i> "		Total	Percentage of salt-water form
			Fresh-water	Salt-water		
1947 ...	Sept. ...		26	52	78	67
	Oct. ...		47	53	100	53
	Nov. ...		30	23	53	43
1948 ...	April ...		9	9	18	50
	May ...		71	30	101	30
	June ...		122	47	169	28
	July ...		132	111	243	45
	Aug. ...		16	208	224	93

In 1947, a year of heavy rainfall, both forms were still abundant in houses up till November but 1948 was a year of poor rainfall, and by September *Anopheles* were becoming scarce. It should perhaps be pointed out that the high catches in June and July 1948 were mainly the result of intensive collecting and the fact that a higher proportion of the catches were tested for exact identity after egg-laying. Although the figures for September, October and November 1947 were based on fewer catches and smaller test samples, so far as could be judged *gambiae* was almost as abundant in these three months as in June and July 1948.

Salt-water *gambiae* was the dominant form during the dry months of September 1947 and August 1948 but the reverse was the case in May and June 1948 when the fresh-water form was dominant. In the catches for the other months recorded in the Table neither form showed marked dominance.

These figures give a rough idea of the comparative abundance of the two forms resting in African village houses, but they give rather a misleading picture of the total population, or even of the blood-feeding activity inside these houses. Although salt-water *gambiae* appears to be highly domestic from the number of blood-fed females found inside houses by day, the number caught represents only part of the total feeding the previous night. The number of salt-water *gambiae* which leave the house after feeding is much greater than that of fresh-water *gambiae*, so that the day-time catch underestimates the incidence of the salt-water form. That is why the mosquito population of houses near salt ponds where dense breeding of salt-water *gambiae* occurs, is so often surprisingly low.

Physiological methods of distinguishing adults is reasonably accurate for estimating the house population provided both forms of *gambiae* lay eggs with equal

facility in the laboratory. But fresh-water *gambiae* lays rather more readily than the salt-water form, and this again is a factor which would tend to underestimate the numbers of salt-water *gambiae* present.

Natural Infections of fresh-water and salt-water *A. gambiae* with Malaria Parasites and *Filaria* Larvae.

Ideal conditions existed in Changombe for comparing the infectivity of the two forms of malaria parasites, as both forms occurred together in the same village houses and were presumably exposed to chances of infection as equal as it is possible to find in nature. In this way Changombe fulfils the same purpose in East Africa as the village of Apapa Lemu did in West Africa when comparing *gambiae* and *melas*.

Wild-caught females were kept in the laboratory until they laid eggs—usually two or three days after capture. The salivary glands were then extracted by Shute's method, and examined for sporozoites, all dissections being made by the author. The identity of the mosquitos dissected was established a few days later when their newly hatched larvae were tested with salt solutions.

In 1947 all the identifications were based on this test and all the adults with four-banded palps proved to be salt-water forms, in Changombe at least. Accordingly, in the following year, four-banded forms were treated as salt-water *gambiae*, while three-banded forms were tested by the physiological method described above.

The results of all dissections are shown in Table III.

TABLE III.

Natural infectivity of salt-water and fresh-water *A. gambiae* with malaria parasites. Changombe, 1947–48.

		Number dissected	Glands positive	Sporozoite rate
Fresh-water <i>gambiae</i>				
Identified by salinity tests in lab.	{ 1947	103	9	
	{ 1948	344	33	
	Total	447	42	9.4%
Salt-water <i>gambiae</i>				
Identified by salinity tests in lab.	{ 1947	128	0	
	{ 1948	404	6	
4-banded forms	1948	209	0	
	Total	741	6	0.8%

Under the same conditions there is a wide difference in infectivity with malaria parasites, fresh-water *gambiae* having a sporozoite rate over ten times that of the salt-water form.

It will be noticed that no gland infections were found among the 209 four-banded salt-water *gambiae* in 1948, while in the same year six infections were found in three-banded salt-water *gambiae*, diagnosed by the physiological test. At first sight this appears a significant difference, but there is evidence to show that the additional dark band on the palps is more clearly represented in the young than in the older females, the dark scales being very easily rubbed off. The difference in infectivity among the salt-water *gambiae* is probably due therefore to selection of a young population, and does not affect the combined figures for all salt-water *gambiae*.

of the mosquitos at this stage might be influenced by the intensity of light inside the hut during the day, one hut was provided with a single window, and the other hut with two windows, one at each end so that the inside of the hut was almost light enough to detect *Anopheles* resting on the ceiling during the day. The huts were visited every morning, the window cage detached and replaced with a new one, and the resting *Anopheles* inside the huts hand-caught by two trained African collectors. Hand catching is now recognised as being a much less efficient method of estimating the mosquito population of houses than spraying with insecticide, but

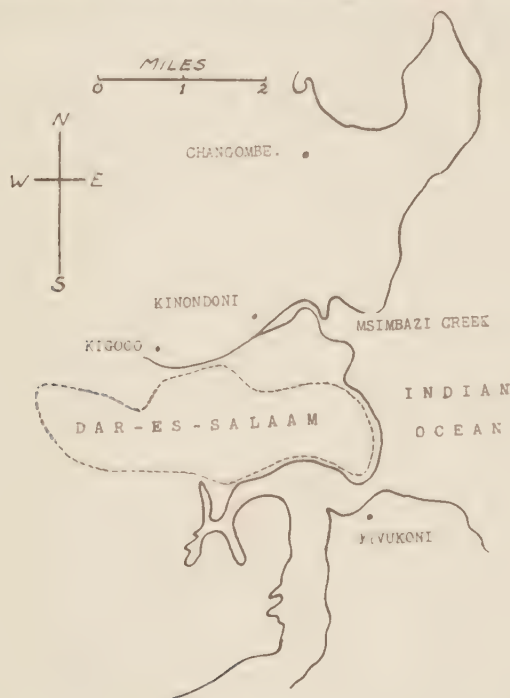


Fig. 1.—Map of Dar-es-Salaam and environs showing localities mentioned in text.

the error was counteracted as far as possible by brushing lightly round walls after the first collection, and repeating the search five or ten minutes later. Any question of house spraying is ruled out when the normal behaviour of mosquitos is to be studied.

One series of experiments was carried out in a pure fresh-water *gambiae* and *funestus* area. The results are shown in Table V.

TABLE V.

Behaviour of blood-fed fresh-water *A. gambiae* in experimental huts with window traps. Kigogo.

Number of observations	Hut with 1 window trap	
	Blood-fed females in hut	Blood-fed females in window trap
44	173	1
16	194	6
37	164	Hut with 2 window traps
		3

It will be seen that fresh-water *gambiae* show very little tendency to leave the hut at dawn after feeding, even over a fairly wide range of illumination inside the hut during daytime, only about 2 per cent. being found in the window trap in all collections. There were still plenty of dark corners to rest in when two window traps were present, and it is possible that when the illumination inside houses by day is very bright, as in most European houses, there may be a stronger tendency on the part of the blood-fed females to leave the house, in the absence of dark daytime resting places. But as far as the ordinary African village house is concerned, it appears that the majority of fresh-water *gambiae* remain indoors on the day following the blood feed.

The results with blood-fed *funestus* are shown in Table VI.

TABLE VI.

Behaviour of blood-fed *A. funestus* in experimental huts with window traps. Kigogo.

	Number of observations	Blood-fed females in hut	Blood-fed females in window trap
1 window trap	24	137	4
	16	66	1
2 window traps	40	46	1

Here also there seems to be little tendency for blood-fed females to leave the shelter of the African hut at dawn after feeding, little over 2 per cent. being found in the window trap. The figures from the hut with two window traps are not very imposing, but suggest that the behaviour is much the same in the better illuminated hut.

The behaviour of these two Anophelines is in striking contrast to that of the dominant Culicines in this area, *Taeniorhynchus (africanus)* (Theo.) and *uniformis* (Theo.), numbers of which were taken in the window trap every morning. Out of 411 blood-fed *Taeniorhynchus* taken in all collections, 331 or 81 per cent. were taken in the window cage, showing the marked tendency of these species to leave the hut after feeding. The day-time catch inside the hut in this case gives no indication of the number feeding there at night.

The second series of experiments was carried out at Changombe where a mixed population of salt- and fresh-water *gambiae* existed. The experiment was run for two months—October 1947 and May 1948—and the figures for the huts with one and two window traps are combined. The proportion of the two forms in the house population was based on a large number of physiological tests.

The results are shown in Table VII.

TABLE VII.

Behaviour of mixed population of fresh-water and salt-water *A. gambiae* in experimental huts with window traps. Blood-fed females. Changombe.

	Number of observations	Blood-fed in hut	Blood-fed in window cage	Percentage salt-water forms in hut
October, 1947 ...	18	135	41	53
May, 1948 ...	20	569	112	30

In this area there is a great increase in the proportion of blood-fed *gambiae* leaving the huts after feeding. Samples of blood-fed females caught in the window trap have all proved to be salt-water *gambiae*, and in view of the finding above that only

about 2 per cent. of the fresh-water *gambiae* leave the hut after feeding, it is reasonably certain that practically all the blood-fed females caught leaving the hut are in fact salt-water forms. As the composition of the house population is known, the proportion of salt-water *gambiae* that leave the hut after feeding can be approximately worked out.

The revised figures are shown in Table VIII.

TABLE VIII.

(Revised from Table VII.)

Behaviour of blood-fed females of salt-water *A. gambiae* in experimental hut.

	Blood-fed females in hut	Blood-fed in window trap	Percentage blood-fed leaving hut
October, 1947...	72	41	35
May, 1948 ...	170	112	40

These figures show that more than one-third of the salt-water *gambiae* feeding inside African houses at night, leave the house at dawn. In this respect, therefore, there is a striking difference in behaviour between salt- and fresh-water *gambiae*.

Half-gravid and gravid females.—As described in a previous report (Muirhead Thomson, 1948) *A. gambiae* in West Africa normally takes 2 days to digest its blood meal and develop its ovaries. This has also been found to apply to the two forms on the East African coast. Suppose for example *gambiae* feed on Monday night. On Tuesday morning nearly all the fresh-water *gambiae* (and *funestus*) remain resting indoors, while about one-third of the salt-water forms leave the hut. On Tuesday evening the females are now classed as half-gravids, many of which leave the house at sundown. Those still left in the house will all be gravid by Wednesday, and leave the house at sundown to lay their eggs.

Trap cages were fitted over the windows before dusk in order to study the movements of half-gravid females in the experimental huts. About one or two hours after dark the window traps were removed, and collections of half-gravid females made inside the hut. It was only possible to carry out this experiment in the pure fresh-water *gambiae* area, the results of which are shown in Table IX.

TABLE IX.

Fresh-water *A. gambiae*. Exodus of half-gravid females from hut at dusk.

	Hut with one window trap	Hut with two window traps
Half-gravid females remaining in hut on night after feeding	14	67
Half-gravid females leaving hut at dusk...	14	32

The results indicate, as far as they go, that up to one-third of the half-gravid females leave the house on the night after feeding, even though they will not be ready to oviposit for a further 24 hours.

Unfortunately there is no information about salt-water *gambiae* at this stage. Using the same technique in West Africa (Muirhead Thomson, 1948) it was found that approximately 70 per cent. of the half-gravid *melas* females left the shelter of the hut at sundown, indicating a much greater exodus than with fresh-water *gambiae*.

Ordinarily, at this time of the day there is a great deal of activity and disturbance in the African village house, and the proportion of half-gravid females leaving the house may on occasions be much larger than the figures indicate. Standardised methods, however, might yield further differences in behaviour between half-gravid females of the two forms, such as has been shown for blood-fed females.

The fact that some half-gravid fresh-water *gambiae* females remain inside the house on the night after the blood meal, while others leave the house, provides an opportunity for finding out if the difference in behaviour is due to differences in age or infection rate. It may be that the younger mosquitos are more restless, or that the older, and possibly infected, females feel the urge to leave the house. This question was put to the test by routine dissections. The results are shown in Table X, and reveal a high gland infection rate in both groups, showing no marked difference in behaviour between infected and uninfected females.

TABLE X.

Fresh-water *A. gambiae*. Infectivity of half-gravid females with malaria parasites.

	Half-gravid females remaining in hut on night after feeding	Half-gravid females leaving at dusk on night after feeding
Number dissected ...	156	84
Glands positive ...	17	6
Sporozoite rate ...	10.9%	7.1%

Outside Resting Places of *Anopheles*.

The observations in experimental huts have shown that with fresh-water *gambiae* there is little tendency for blood-fed females to leave the shelter of the hut at dawn after feeding. But on the following night, when the blood is half digested and the ovaries half developed, roughly one-third of the half-gravid females leave the shelter of the house, even though it provides ideal resting places. It might be expected, therefore, that this stage at least would be found resting out of doors.

In West Africa blood-fed and gravid *gambiae* were taken regularly in outside resting places, success being due to the fact that resting mosquitos were highly concentrated in a particularly suitable stretch of heavily shaded earth bank.

In and around Dar-es-Salaam no such ideal location has been found, so many suitable outside resting places being present in the experimental areas that the task of searching for the odd *Anopheles* in the innumerable thickets has proved too formidable. Previous attempts to find Anopheline resting places out of doors on the East African coast have not been very successful. In a wide range of potential resting places Wiseman and others (1939) recovered only 3 female *gambiae*, the condition of which was not recorded.

This problem has been dealt with in another way which has proved much more satisfactory, and infinitely less laborious. In the Kivukoni area south of the harbour, little horizontal tunnels or pits were dug in shaded earth banks, providing about half a dozen dark shelters each about one cubic foot in volume. In this way small numbers of *Anopheles* could be found where none was recovered outside before. In these collections unfed females have been ignored, and the figures refer only to those which have had at least one blood meal.

The results are shown in Table XI.

TABLE XI.

Anopheles found in out-door resting places. Kivukoni.

Number of observations	Fresh-water <i>gambiae</i>		" <i>funestus</i> "	
	Blood-fed	Gravid	Blood-fed	Gravid
21	4	21	34	51

Many more gravid female *gambiae* than blood-fed were found resting outside as was to be expected. The results with *funestus* are rather unexpected. Observations in the experimental huts at Kigogo on the north-east fringe of Dar-es-Salaam had indicated that *funestus* like fresh-water *gambiae* showed little indication of leaving the house after feeding. In Kivukoni, on the other hand, the proportion of blood-fed *funestus* leaving experimental huts was much higher than at Kigogo. The blood-fed *funestus* found in these outside shelters may have come from houses, or they may possibly be females which had fed out of doors. A further difficulty is that the *funestus* group is not an easy one, and although none of the females examined appeared to belong to one of the other closely allied species, there was such considerable variation in the width of the dark band on the palps that some of them may not have been *funestus* at all.

In the case of salt-water *gambiae* it would have been interesting to link up the house population with that in outdoor resting places, in the same way as was done with fresh-water *gambiae*. Several little shaded pits were constructed at Changombe to sample the outside population, but they did not prove very attractive in that area. A much more suitable outside shelter was discovered accidentally.

In the construction of experimental huts the African builder had taken all his earth from one large pit about 8 ft. deep and about 5 ft. diameter. By undercutting the vertical walls all round, a few feet from the bottom, heavily shaded resting places were provided and 4 blood-fed and 2 gravid salt-water *gambiae* were found resting in this pit. The mosquito population in nearby houses was very low by this time, otherwise much larger samples might have been obtained. This method, however, seems to have great possibilities for sampling not only the mosquito population resting out of doors, but also for studying those mosquitos which normally feed out of doors, and are seldom taken in houses.

Salinity as a limiting Factor in salt-water *A. gambiae* Breeding, and its possible bearing on Control.

Previous records in Dar-es-Salaam (Mackay, 1938) have shown that larvae of *gambiae* were taken over a wide range of salinity, up to 15.7 gm. chloride per litre (i.e. 80 per cent. sea water, taken as containing 19.00 gm. per litre), and that within that range development can be completed. The same author says that "*A. gambiae* var. *melus*" can withstand chloride concentrations up to 19.8 gm. per litre.

In Mauritius the high degree of tolerance of salt-water *gambiae* to salinity was shown by Gebert (1936) who found that larvae could develop from egg to adult at a salinity of 24.55 gm. NaCl per litre (i.e. about 78 per cent. S.W.). More recently Jepson & others (1947) report that *gambiae* can be made to pass through several stages in pure sea water, and that in one salt pan larvae were found in a slightly viscous salt solution at 65 gm. NaCl per litre.

Although larvae can occasionally be found at such high salinities, it does not necessarily follow that complete development was carried through, or was even possible under such conditions, as salinity may increase very rapidly in evaporating

pools. In the same way laboratory findings on the maximum salinity at which complete development from egg to adult can take place, may give a misleading picture of what happens under natural conditions.

An unusual opportunity occurred in the present investigation of finding out at what point increasing salinity becomes a limiting factor for development of salt-water *gambiae* in the field.

Near Dar-es-Salaam continuous dense breeding of salt-water *gambiae* occurred in certain shallow salt ponds up to half a mile inland from the tidal zone, during the months of September and October 1947. Large numbers of larvae and pupae were taken regularly from one of these ponds to provide material for experiments. During that time the salinity was 11-12 gm. Cl. per litre, *i.e.* about 60 per cent. sea water. In early November, after a long dry spell, larvae became exceedingly scarce even though there was still plenty of water in the pond, and its appearance was unchanged. The salinity was then found to have increased to 16 gm. per litre, *i.e.* about 83 per cent. sea water, and subsequently not a single larva was found. Within a week the salinity of the salt pond had reached that of sea water, and it was completely free of all larvae, including those of the dominant Culicine, *Culex sitiens* Wied., which had been enormously abundant up to that time. Nearby fresh water ponds at the same time were shrinking in volume and becoming brackish, with up to 15 per cent. sea water, and in these large numbers of salt-water *gambiae* larvae were appearing for the first time.

As the experiment was quite unplanned it is not possible to say whether the disappearance of breeding from this very favourable pond was due to development of larvae being hindered, or to cessation of egg laying, but it does provide a clear demonstration that continuous breeding of salt-water *gambiae* is no longer possible when the salinity reaches about 83 per cent. sea water.

The limiting factor to salt-water *gambiae* breeding provided by increasing salinity explains the rather restricted nature of its breeding grounds round Dar-es-Salaam. There are no large rivers pouring great volumes of fresh water into the sea at this point, and there are no true estuarine conditions such as exist on other parts of the Tanganyika coast, *e.g.* the Rufiji Delta. As a result the salinity of the water of the Indian Ocean remains high throughout the year. Normally it fluctuates between 19.8 and 20.8 gm. Cl. per litre (figures which are actually higher than the 19.00 gm. per litre usually selected as the arbitrary standard for 100 per cent. sea water). Even at the end of the rainy season, the lowest figure recorded was 18.8 gm. Cl. per litre. This means that at high spring tides the intertidal area is flooded with water the salinity of which is too high to permit salt-water *gambiae* to breed and unless there is considerable dilution with fresh water from the land, conditions will remain unfavourable.

Reference has already been made to the large *Avicennia* orchard which occupies the main tidal part of Msimbazi Creek, in which no indication of salt-water *gambiae* breeding was found during the favourable season of 1947. It now seems fairly certain that the high salinity of the spring tide water flooding this ground was the limiting factor, and that the greatly reduced flow of water in the Msimbazi River was unable to keep the salinity sufficiently low for breeding to take place. In the same way the continued absence of salt water *gambiae* from salt pans in this area can be attributed to the same factor.

The high salinity of the Indian Ocean along the Dar-es-Salaam coast suggests a method of dealing with a local problem of intense Anopheline breeding. Kivukoni, the area south and east of the harbour, has long been recognized as a major source of *Anopheles*, and although some control work has been carried out in recent years, there still remain two extensive fresh water swamps which are quite uncontrolled.

The position of these in relation to the residential quarter of Dar-es-Salaam is shown in fig. 2 and Plate XI, fig. 2.

Houses beside these swamps may have anything up to 100 *funestus* per room, and there may be a high production of *Anopheles* throughout the dry season, as happened in 1947-48. Adult *funestus* can be taken regularly in African houses on the water front, just across the harbour mouth from the residential quarter, and it is clear that such vast breeding grounds near the town cannot be ignored.



Fig. 2.—Map showing fresh-water swamps south of Dar-es-Salaam harbour.

In one of these swamps the shoreline is enormously extended by long ridges of sweet potato plots projecting into the water edge. The narrow trenches between the ridges are flooded with swamp water, and provide ideal breeding places for *funestus*.

Exact data as to the level of these swamps in relation to mean tide level, or as to their depths, has not been available, but the general opinion is that they are too low to be drained. These swamps are mainly catchment areas, and there are no rivers flowing into them. From the map it can be seen that one swamp is about 300 yds. from the sea, and that the two swamps are separated by about 500 yds. of dry land.

It is suggested that these two swamps should be connected to the sea by deep channels in such a way that impounded water flows out at low tide, and sea water enters at high tide. By means of a suitable arrangement of tidal gates it should be possible for the sea water to circulate in the swamp, entering at one end at high tide, and being released at low tide at the other end. *Finestus* and fresh-water *gambiae* would be completely eliminated in this way; and as the salinity of the water increased towards that of sea water, any possibility of salt-water *gambiae* becoming established would be ruled out.

The elimination of *Anopheles clutus* Edw. from Durazzo Marsh (Hackett, 1937) is an excellent illustration of how successful and permanent such a method can be.

Discussion.

The facts assembled in this paper show that salt-water *gambiae* in East Africa is not the same as *Anopheles melas* in West Africa and that these salt-water forms have evolved quite independently on the two coasts. This is not altogether surprising as the finding of *A. melas* on the East African coast would have raised some awkward problems of discontinuous distribution. In West Africa the distribution of *A. melas* is closely linked, in estuarine regions at least, with the nature of the mangrove belt, and in particular with the occurrence of large *Avicennia* "orchards". The mangrove belt in West Africa is dominated by two forms, *Avicennia* and *Rhizophora*, and shows close agreement with mangroves on the other side of the Atlantic (Chapman, 1944). On the East African coast, however, the mangroves are more akin to those of the Old World. In addition to *Rhizophora* and *Avicennia*, there are several other genera such as *Sonneratia* and *Bruguiera*, that are not represented on the Atlantic coasts. There is no continuity between the mangroves of East and West Africa, and *melas* has evidently not succeeded in rounding the Cape.

The exact relationship between fresh-water *gambiae* and salt-water *gambiae* remains to be seen. As all attempts at cross-fertilisation failed under conditions where negative results could not be considered significant, our knowledge remains much less complete than it is with *melas*. The rôle of salt-water *gambiae* as a vector of malaria has been established, and although it appears of minor importance compared with typical *gambiae*, there may be places on the East African coast, or in Mauritius, where it would have to be treated more seriously.

In studying the habits of these *Anopheles* in houses, the technique evolved in West Africa has again proved invaluable, especially in assessing the mosquito population which leave the shelter of the house. What happens to those mosquitos once they have left the house is still not perfectly clear. In the present report it has been assumed that once having left the house the majority remain resting outside. This is supported by the fact that specimens of *gambiae* are found in outdoor resting places; in such places the majority of the females are gravid, which agrees very well with the finding that the half-gravid females leave the shelter of the hut to a much greater extent than those freshly blood-fed.

On the other hand there is plenty of evidence to show that in the latter part of the 2-3 day gonotrophic cycle some *gambiae* return once more to the shelter of the African house. Although there is no sign of this happening in the type of experimental hut used in the present experiments, the ordinary African house offers much more obvious wide openings which would appear as attractive dark resting places.

Haddow (1942) in his hut experiments on the shores of Lake Victoria found a peak of entry at dawn mainly due to entry of gravid females. In his huts there was a 1 ft. gap between the walls and eaves, and in addition the huts were built under the shade of a large fig tree. Hocking & MacInnes (1948), using spraying technique, concluded that females left and entered huts in approximately equal numbers each night, and that the later stages in the gonotrophic cycle rest almost entirely in huts and not in the open bush.

It is possible that these conflicting findings may be due to difference in technique, housing, or local conditions. The experience of the writer in both West and East Africa indicates that *gambiae* makes use of outdoor resting places to a considerable extent during its gonotrophic cycle, and that many of the females leaving houses before the cycle is complete do not return to the shelter of the house. What proportion this represents it is difficult to say, but it may prove to vary considerably in different parts of Africa, depending on climate or suitability of outdoor resting places.

Summary.

The brackish water form of *A. gambiae* on the East African coast and probably in Mauritius—is not the same as *A. melas* of West Africa.

In salt-water *gambiae* a variable proportion of the females have an additional dark band on the palps, resembling 4-banded *melas*, but the remainder are indistinguishable from typical *gambiae*.

Eggs and larvae of salt-water *gambiae* show no morphological differences from those of fresh-water *gambiae*, thereby differing from *A. melas* of West Africa.

Larvae of the two forms show a clear-cut difference in reaction to sudden changes in salinity, and a simple test has been worked out whereby wild-caught females can be accurately identified by the reactions of their progeny.

This physiological test has formed the basis of all work in comparing the incidence, habits, and infectivity of salt and fresh-water *gambiae* in Dar-es-Salaam.

Exposed to equal chances of infection in the same village during 1947 and 1948, fresh-water *gambiae* had a sporozoite rate of 9.4 per cent, while that of salt-water *gambiae* was 0.8 per cent.

About 4 per cent. of both forms were infected with filaria larvae, but monthly figures showed that infection rates in salt-water *gambiae* may rise to 22 per cent.

Fresh-water *gambiae* show little tendency to leave African houses at dawn after feeding, whereas in salt-water *gambiae* over one-third of freshly blood-fed females leave the house at dawn.

In fresh-water *gambiae* many half-gravid females leave the shelter of the house at dusk on the night after the blood feed. There is no marked difference in infectivity between those which leave the hut and those which remain indoors at this stage.

Blood-fed and gravid females of fresh-water *gambiae*, *funestus*, and salt-water *gambiae* have been found in outdoor resting places, gravid females predominating in the case of the first two.

Although larvae of salt-water *gambiae* can complete their development in pure sea water, in nature increasing salinity becomes a limiting factor before it reaches that of sea water, continuous breeding being no longer possible at salinities over 83 per cent. sea water.

Salinity as a limiting factor explains the rather restricted breeding of salt-water *gambiae* on the coast, and suggests that certain coastal fresh-water swamps at Dar-es-Salaam could be cleared of all Anopheline breeding by salinifying with sea water.

References.

- CHAPMAN, V. J. (1944). J. Linn. Soc. Lond. (Bot.), **52**, no. 346 p. 407.
- GEBERT, S. (1936). Trans. R. Soc. trop. Med. Hyg., **30**, pp. 255-257.
- HACKETT, L. W. (1937). Malaria in Europe.—336 pp. London, Oxford Univ. Pr.
- HADDOW, A. J. (1942). Bull. ent. Res., **33**, pp. 91-142.
- HOCKING, K. S. & MACINNES, D. G. (1948). *Ibid.*, **39**, pp. 453-465.
- JEPSON, W. F., MOUTIA, A. & COURTOIS, C. (1947). *Ibid.*, **38**, pp. 177-208.
- MACKAY, R. (1938). Second (Final) Report of the Malaria Unit, Dar-es-Salaam, 1934-1936.—61 pp. Dar-es-Salaam.
- MUIRHEAD THOMSON, R. C. (1945). Bull. ent. Res., **36**, pp. 185-252.
- MUIRHEAD THOMSON, R. C. (1948). *Ibid.*, **38**, pp. 527-558.
- RIBBANDS, C. R. (1944). Ann. trop. Med. Parasit., **38**, pp. 85-99.
- TREDRE, R. F. (1946). *Ibid.*, **40**, pp. 380-420.
- WILSON, D. Bagster. (1936). Report of the Malaria Unit, Tanga, 1933-34.—71 pp. Dar-es-Salaam.
- WILSON, D. Bagster. (1946). E. Afr. med. J., **23**, pp. 258-272.
- WISEMAN, R. H., SYMES, C. B., McMAHON, J. C. & TEESDALE, C. (1939). Report on a Malaria Survey of Mombasa.—60 pp. Nairobi.
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FIG. 1. Brackish water breeding places of salt-water *gambiae*.



FIG. 2. Aerial photograph of Kivukoni, the area south and east of Dar-es Salaam harbour, showing freshwater swamps. (See Fig. 2).

A COMPARATIVE METHOD FOR TESTING AGENTS USED IN SHEEP MAGGOT FLY CONTROL.

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Dipping or spraying sheep with various agents has for long been the principal method of maggot fly control. Although much critical and fundamental work has been carried out, the assessment of the degree of control and the length of protection afforded, has depended ultimately on field trials. There are, unfortunately, several complicating factors associated with the problem which in the past have received insufficient attention. They have often resulted in conclusions being drawn from field work which are either invalid or are not supported by adequately controlled experiments.

MacLeod (1938) drew attention to some of the intrinsic difficulties, and criticised previous field trials on three main points: (1) "The low incidence of strike which can be expected under natural conditions during the normal period of a trial". To this must be added the fact that only a limited number of sheep can be accurately observed, and that it is not unusual for the incidence of fly-strike to drop practically to nil for periods of three weeks or longer. (2) "The variation in individual susceptibility of sheep". In order to attain truly identical conditions for controls and treated sheep, the numbers must again be limited, thereby emphasising the importance of individual variations. This point has recently been reiterated by Stamp, Watt and Beattie (1948) with particular reference to the susceptibility of "scouring" sheep to crutch strike. (3) "The somewhat paradoxical fact that the development of strike may not be a true index of the failure of a dressing". Maldwyn Davies and Hobson (1935) have shown that the development of strike from blow is largely dependent on fleece humidity, and that the humidity in the normal fleece is usually unsuitable for such development. It follows that the majority of blows which occur on sheep are often prevented from completing their development owing to purely physical conditions, yet the presence of these blows may be an important indication of the failure of a dressing. This particular aspect has become very much more important with the introduction of the newer insecticides. For example, DDT is known to act primarily against the adult fly and thus prevent oviposition; it has little or no larvicidal action. To test the properties of such an agent by using strikes as the criterion is obviously unsound.

One further source of error has been indicated by Hughes, Jenkins and Jones (1946) and others. It has been observed that when control sheep are grazed in company with DDT dipped sheep, the incidence of strike on the controls is lower than would be expected. There are grounds for suggesting that the action of DDT on the "fly" population has resulted in a partial protection of the control sheep. It will be appreciated that this effect is confined to agents which act against the adult fly ("anti-adult"), and does not occur with larvicidal agents.

In an attempt to overcome some of these difficulties MacLeod made use of small groups of carefully selected and closely observed sheep. An "attractant", developed by Hobson (1936), was applied to the sheep and this had the effect not only of increasing the incidence of blow, but also of rendering all the sheep more or less equally attractive to the adult fly. By observing the occurrence of blows on these sheep, MacLeod was able to assess the "anti-adult" value of the preparation under test. To assess larvicidal value a larval implantation technique was employed.

This line of investigation represented a considerable advance, in that it did take into account some of the sources of error already outlined. A real attempt was made to differentiate the various modes of action of the preparations employed, by using separate tests to demonstrate "anti-adult", larvicidal and ovicidal properties. The older methods had merely given results in terms of length of protection without specifying precisely against what the sheep were being protected. The published results from the use of MacLeod's technique are unfortunately not extensive, and it is difficult therefore to judge its true practical value. One shortcoming is obvious. In spite of the use of an "attractant" to increase the incidence of blow, the worker is still at the mercy of the natural variation in "fly" activity; the absence of such activity might well invalidate the results of a trial for periods of three weeks or more. As will appear later, some criticism can also be levelled at MacLeod's technique of larval implantation.

The idea that the variation of natural incidence could be overcome by releasing artificially bred maggot flies into a fly-proof sheep chamber originated in Australia. A chamber of this type is described in the First Report of the Joint Blowfly Committee (Tillyard & Seddon, 1933), and has apparently been used "entirely for experiments in the induction of strike in living sheep" but, so far as the writer is aware, no technique for the testing of protective dips by this method has been reported. Downing and Froggatt (unpublished work, 1942), also in Australia, constructed a "Fly-Sheep Chamber" and by making use of the constant and dense "fly" population which it provided, were able to carry out some preliminary work on the testing of dips, sprays and other dressings. The present paper deals with the development of a "Fly-Sheep Chamber" technique in Britain and its use in a critical study of the protective properties of certain agents used against the sheep maggot fly, *Lucilia sericata* (Mg.). Whilst this technique has proved most valuable for testing the "anti-adult" properties of dressings, it has not always been possible to maintain conditions suitable for the development of strikes. A modified larval implantation technique has therefore been employed for assessing larvicidal properties, this being used in conjunction with the first technique to provide a comprehensive test.

This paper is divided into two parts, the first of which is confined purely to a description of the technique employed for testing the properties of protective dips and sprays. In the second part three series of results are presented which serve to illustrate the working of the technique, and also give some indication of the relative merits of several agents commonly used for maggot fly control.

It is important to emphasise at the outset that the present techniques are not intended to reproduce conditions which are identical with those occurring in nature. For this reason no attempt is made to estimate the actual length of protection which is given by any particular agent, but only to compare its protective value with that of some other agent. It is appreciated that such factors as urine staining and "scouring" have a most important bearing on the problem in the field, but in order to establish the comparative fundamental values of different agents it is essential that as many complicating factors as possible should be eliminated.

Materials.

The "Fly-Sheep Chamber". (F.S.C.)

The term "Fly-Sheep Chamber" is used to refer to a fly-proof building, in which sheep can be exposed to an active and dense fly population, maintained under reasonably well controlled conditions of temperature and light. The development of such a chamber, in which flies could be relied upon to behave in a fairly normal manner and to pay due regard to the sheep, did not prove to be simple. For the sake of brevity, however, a description will only be given of the final arrangement.

The *structure* was adapted from a large well-house, octagonal in shape, with a slate roof and open sides, the diameter between opposite sides being 23 ft. The open sides were filled in with a double layer of "Windolite", a transparent celluloid type of material, and a plaster-board ceiling 8 ft. in height was fitted. A cement floor with suitable drainage was laid. Internally a pen, of posts and sheep wire, was erected, leaving a surround of 3 ft. between the pen and the sides of the building. Further posts with jointed hurdles, provided a convenient catching pen and "race", for handling and examining the sheep.



Fig. 1.—External view of Fly-Sheep Chamber.

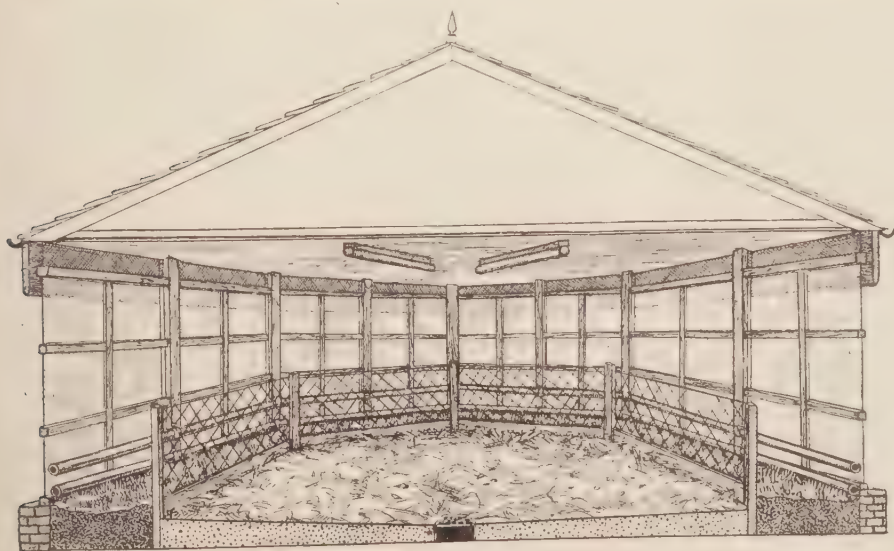


Fig. 2.—Diagram of internal arrangement of Fly-Sheep Chamber.

Heating.—The F.S.C. is heated by a hot water system extending round all the sides. During the summer months artificial heating is not usually necessary, since the optimum working temperature lies between 65° and 80°F., and a fall of temperature below 65° is permissible during the night. Variations of temperature within the above limits has not been found to have any important effect on "fly" activity, but a working temperature of 70°–75° is probably the most suitable.

The *lighting* of the centre of the chamber is important, and is provided by four 40-watt "daylight" fluorescent tubes arranged in a square on the ceiling. These lights give an even illumination of the sheep's backs which is necessary for normal fly activity and provide a counter attraction to the daylight coming in through the windows of the chamber. *Lucilia sericata* exhibits marked phototropism, and it is found in practice that the flies tend to congregate on that side of the chamber having the greatest light intensity. For this reason it is considered essential that the chamber should be almost circular in shape, in order to eliminate bright corners in which the flies otherwise tend to congregate.

Humidity.—No true humidity control is maintained in the F.S.C. owing to the elaborate equipment which would be required to obtain a suitable level in combination with a high temperature and adequate ventilation. It is probable that this lack of high humidity is responsible for the low rate of development of blows to strikes which frequently obtains, but since the F.S.C. is used only as a test of "anti-adult" properties the point is not considered of any great significance. In practice an attempt is made to maintain humidity by frequent watering of the surround area between the pen netting and the walls of the chamber. This space is filled with soil and a green crop such as mustard is cultivated, with a view not only to raising the humidity, but also to provide a natural resting site for the flies.

Ventilation is obtained by a gauze panel 9 ins. wide extending right round the building at the junction of the sides and ceiling; this space is baffled against wind by an outside weather board. During hot summer weather the circulation of air obtained in this way is barely adequate, and a rather high concentration of ammonia develops within the chamber. Later in the season, on the other hand, it is often necessary to block up part of this ventilation space in order to maintain a correct temperature.

Flies and their breeding.

The choice of the species of maggot fly to be used in the F.S.C. must naturally be determined by the problem under investigation and the country in which the work is being carried out. In Great Britain it is generally accepted that the primary fly which is responsible for over 90 per cent. of natural strikes, is *Lucilia sericata* and for this reason, and in order to avoid unnecessary complications, this species is used throughout.

A full account of the breeding technique is outside the scope of the present paper, but a few details of the production of flies for use in the F.S.C. are desirable. Clusters of eggs, or blows, are taken as required from a series of cages containing a pure strain of *Lucilia*. The egg masses are sub-cultured on pieces of raw lean beef where hatching and feeding of the maggots take place. The fully fed third-stage larvae leave the meat in from 3 to 4 days and burrow into an underlying layer of sand and sawdust from which they are later sifted and transferred to clean dry sand and sawdust. Pupation takes place in from 4 to 6 days, and after this time the pupae are separated from the sand mixture and placed in jars; prepupae which are slow in pupating are discarded. The pupae are incubated at 80°F. until flies start to emerge; the jars of pupae are then transferred to large cages, measuring 30 ins. by 30 ins. by 30 ins., in which the population is allowed to build up to approximately 2,000. On an average this is completed in two days during which time the flies are provided

with a diet of dry sugar and water. Excess pupae are removed from the cage and the flies are now ready to receive a protein meal.

Before the adult female fly can become fertile, it is essential that she should receive at least one full meal of animal protein and for this, raw ox liver has been found to be the most suitable. A large tray of incised liver is therefore placed in the cage and the flies are allowed 24 hours in which to feed. The liver is then removed and after an interval of 48 hours a fresh feed is provided. It is usually found that a number of flies will blow on this second feed in which case the liver is removed, but should they not do so, a third feed is given 24 hours later and removed at the first indication of blow. At this stage one can assume that the majority of the female flies are becoming capable of laying fertile eggs. By holding the flies for a further two to three days without access to protein food, a high potential of blow is assured, and in this condition the flies are ready for use in the F.S.C.

The breeding and "conditioning" of the flies is so arranged that one complete cage will be available on each occasion when sheep are due to be exposed in the F.S.C. In practice a considerable population will normally be present in the chamber from the previous exposure, but in order to guarantee that a high potential of blow is available, at least 2,000 fresh flies are always added to the number each time a batch of sheep is exposed. Inside the chamber water and dry sticks of sugar are available as a maintenance diet for the flies.

The sheep.—Yearling Romney-Southdown cross-bred sheep have been used as the standard type throughout the present work. It was observed, in an early experiment, however, that some of these sheep were not of identical wool type. They could, when in full fleece, be roughly divided into two groups which approached more or less the parental types, that is, a close woolled Southdown and a more open woolled Romney. It was further observed that the incidence of blow had been higher on the Romney type fleeces. This variation in wool does not become apparent until some two to three months after shearing. In selecting sheep for experimental purposes, therefore, the practice has been adopted of grading the sheep when in full fleece, and discarding any which show marked variation from the standard type.

The sheep are shorn six weeks prior to the commencement of the experiment, and are divided into groups corresponding with the preparations to be tested, each group containing not less than five sheep. The sheep are branded with a group number on the flank, and in addition carry an individual ear number.

"Attractant."—The work of Hobson (1936) indicated that certain ammoniacal substances assist materially in attracting the maggot fly to sheep and encourage oviposition. Various "attractants" of this type were tested in the F.S.C. but, owing no doubt to the concentration of ammonia already present in the chamber, they did not prove so effective as they do out of doors. Following up the earlier work of Hobson (1935) tests were carried out using the natural exudates produced by feeding maggots. A large piece of raw ox liver was implanted with egg clusters and the resulting maggots were allowed to feed until considerable disintegration of the liver tissue had occurred. By about the fourth day, the whole mass was in a semi-liquid state of decomposition, and this was then transferred to a fine sieve where it was compressed in order to expel the maximum possible quantity of fluid exudate. This fluid was then diluted with glucose saline until it was approximately the consistency of thin cream. It was found that a liver extract of this type was extremely attractive to the maggot fly and that it does not lose its potency when employed under F.S.C. conditions. This "attractant" has therefore been adopted for use in the present tests, and when applied to sheep remains active for several hours; two applications a day are sufficient to maintain the sheep in a highly attractive state for "fly" activity. The efficiency of the method is demonstrated by the fact that any blows which do occur, are invariably found in the region of the

"attractant", and that when this is present no blow has ever been observed elsewhere.

Methods.

Routine of exposure.

The essential feature of the F.S.C. technique is that the test of the protection afforded by the various dips, sprays, etc., is strictly comparative. The groups of sheep are treated respectively with the several agents under tests on the same day, and from that time are dealt with as a single flock. For the greater part of the time they are grazed out of doors, so that the fleece is subjected to normal weathering. Once every week the flock is brought into the F.S.C. for 48 hours, and there exposed to a dense fly population. As has been stated, this population is reinforced by the release of about 2,000 "conditioned" flies whenever the sheep are exposed. It is desirable that the sheep should be fairly dry when they enter the chamber, since it is found that the incidence of blow is less on wet sheep; it is sometimes necessary, therefore, during wet weather to keep the flock under cover for the 24 hours prior to exposure.

The "attractant" is applied to the sheep as soon as they enter the F.S.C., usually about 10.30 a.m., and again at 2.30 p.m.; the process is repeated at the same times on the following day. The site chosen for the "attractant" is always on the back of the sheep, and it is applied to the same identical spot during any one exposure period; from one exposure to the next it is desirable to vary the position. Having parted the fleece at the required point, a teat-ended pipette is used to deliver approximately 1 ml. of "attractant" direct on to the skin, and the wool is then pressed back into its normal position over the site.

At the end of 48 hours the sheep are released from the chamber and are minutely examined for 6 ins. round the site of the "attractant". Blows or strikes which may have occurred are recorded for the individual sheep concerned. Blows are left *in situ* for observation of subsequent development, whilst strikes are dressed with a small quantity of Boro-Glycerine (20 per cent. Boric Ac. in Glycerine), which has the property of eliminating maggots without providing any protection at a later date.

Development of blows to strikes.

Reference has already been made to the work of Maldwyn Davies and Hobson, in which it was shown that a high level of humidity is the chief factor determining the further development of a blow to a strike. These views have been confirmed by several workers in the field who have shown that under normal conditions only about 50 per cent. of well-placed blows develop to strikes. The present work in the F.S.C. has shown that here too, during the summer months, the rate of development of blows on control sheep is approximately 50 per cent.; later in the year this figure drops progressively, until in the late autumn virtually no successful development takes place.

It must be appreciated at this juncture that the "anti-adult" value of an agent, or its power to prevent oviposition, is reflected only in the incidence of blow, whilst larvicidal value is reflected in the development rate of blows to strikes. It has been found that in the F.S.C. the incidence of blow is not subject to any great variation, but that the development of blow to strike is rarely greater than 60 per cent. and may fall to nil. In order to test a larvicide, a high development rate on control sheep is a prerequisite, and it has therefore been realised that, although the F.S.C. provides a reliable test of "anti-adult" properties, it may not always provide a sure test of larvicidal properties. For this reason it has been necessary to develop a separate test for the estimation of larvicidal value.

Development of larvicide test.

The artificial production of strikes with a high degree of certainty, is a problem which has occupied the minds of many workers, and several methods of achieving this object have been described, but for the purposes of a comparative larvicide test all are more or less open to the same criticism.

Freney, MacKerras and MacKerras (1935) described a method which involved the implantation of about 2,000 first-instar larvae on the sheep's skin followed by a thorough wetting of the surrounding fleece. By this technique strikes could apparently be produced with a fair degree of certainty, but it is felt that the implantation of such a large number of larvae direct on to the skin, as opposed to high up in the fleece, represents a state of affairs too far divorced from natural conditions, and is unlikely to provide a fair test. In addition, the thorough soaking of the fleece is an undesirable feature which might well dilute, mask, or activate a larvicide, depending on its nature.

A very much better method was described by MacLeod (1937) after he had made numerous unsuccessful attempts to induce the regular development of strike from naturally occurring blows. The technique involved the application of a little water to the base of the wool, with subsequent production of an erythema of the skin at this point by rubbing with a wet finger over a patch about half an inch in extent. On to this area were placed newly hatched larvae cultured from a blow produced in the insectary. The larvae were then covered with a compact plug of cotton wool which had been soaked in water and the excess lightly squeezed out. Finally, the plug was kept in position by sewing the fleece together over it.

By this method, MacLeod was able to produce strikes at any required point with remarkably few failures even under the most adverse weather conditions, and he used the technique to carry out tests of the protective period provided by arsenic and other dips, finding the effective period for arsenic to be in the region of three weeks.

This technique, though highly reliable from the point of view of production of strike, is open to two serious criticisms as regards a comparative test for larvicides. In the first place, a natural blow does not occur on the skin but well up in the fleece, and therefore the newly hatched larvae on a dipped sheep are forced to penetrate a wool barrier impregnated with larvicide before they can reach the comparative security of their feeding ground on the skin, and this at the most vulnerable time of their larval existence. Secondly, chemical estimation of the DDT and BHC content of various parts of the wool staple have shown that there is little, if any, insecticide present on that part of the fibre which has grown since the date of dipping. Taking then, the period at which MacLeod found arsenic to fail as three weeks, there will be at that time, above the skin, a length of new wool of approximately a quarter of an inch. This wool would be almost entirely free of DDT or BHC and, by inference, of arsenic also. It is into just this area that the first instar larvae are placed by his implantation technique.

It will readily be seen from these observations that there are reasonable grounds for suggesting that the break in larvicidal activity indicated by either of the above techniques may not present a true picture. It may be that the apparent failure of protection is a result of the natural wool growth, leaving a larvicide-free area on the skin in which maggots may flourish, whilst an efficient larvicide may still be present higher up in the staple. What is required for the purposes of the ideal test, is the development of a larval implantation technique which, whilst ensuring a high rate of development of strikes on untreated control sheep, will also allow any larvicide in the fleece of a treated sheep to exert its full effect in a manner which resembles as closely as possible that obtaining under natural conditions.

The technique which has finally been adopted for use in the larvicide test is a modification of MacLeod's method. The fleece is parted in the selected site and four or five drops of water are placed on the skin. The skin at this point is then lightly scratched several times with the finger nail to produce erythema, and a long narrow moistened plug of cotton wool, about 3 ins. long by half an inch wide, is pressed into the parting of the fleece down on to the skin. The fleece is closed over the plug and a cluster of 300-400 newly emerged first instar larvae is placed in the fleece directly above the plug and about half an inch from the fleece tip, this being the site in which naturally occurring blows are found. The wool tips are finally brought together and secured over the cluster of larvae by a small piece of transparent cellophane adhesive tape. After implantation the sheep are turned out normally to graze for twenty-four hours, at the end of which time they are examined to discover whether establishment of the larvae has taken place. It is not advisable that this technique should be used on sheep when the fleece is wet, nor that they should be exposed to heavy rain subsequent to implantation. The whole technique has been carried out successfully indoors when spells of wet weather have persisted.

It has been found that by employing the above method, strikes on untreated sheep can be produced in between 75 per cent. and 90 per cent. of cases, even under adverse autumn conditions and, though this may not be quite such a high degree of certainty as is obtained by MacLeod's method, it does approximate much more closely to the natural development of strike. Amongst the advantages of this method might be mentioned: (1) the natural position in which the larvae are placed, (2) the downward attraction provided for the larvae by the moist plug which also maintains sufficient humidity to prevent their desiccation, (3) their full exposure to any larvicide present in the fleece, (4) the minimum of interference with the wool barrier bearing the larvicide.

The complete test for protective properties.

By using the larval implantation method in conjunction with the F.S.C. technique, it is felt that a complete test is now available which is capable of assigning comparative values to the various protective agents, regardless of whether they exert their effect by virtue of larvicidal or "anti-adult" properties.

The routine adopted consists of the weekly exposure of treated groups of sheep in the F.S.C. until a break in the "anti-adult" protection of any group is indicated by the presence of sufficient blows. During this period a number of blows may develop to strikes, but as has already been observed such development may be largely dependent upon the variable humidity existing in the F.S.C. Further, since the presence of blows is the true index of "anti-adult" properties, it is considered better at this stage to ignore strikes as such and to treat them as having the same significance as blows.

Once a group as a whole has broken down to blows in the F.S.C., i.e., "anti-adult" protection has failed, weekly implantations are carried out to determine whether any larvicidal properties remain which may have out-last the protection provided against the adult fly. It will be appreciated that it is unnecessary to apply the implantation test before a group has broken in the F.S.C., since no maggots can be present on a sheep which is still protected against blow by an efficient "anti-adult" dressing.

Assessment of Results.

Some explanations and definitions are now necessary with regard to the results obtained from the two foregoing tests. So far as the larvicide test is concerned the interpretation is simple. On examining the sheep 24 hours after implantation it is at once apparent whether or not establishment of larvae has taken place—there

are no intermediate stages. If a cluster of larvae is found actively feeding on the skin, this constitutes a strike and, on that sheep, larvicidal protection is deemed to have failed. If on the other hand the larvae are dead or, if living, are scattered in the fleece, then no strike is recorded. Larvae, which are unable to establish themselves on the skin within 24 hours, invariably die.

An examination of sheep which have been exposed in the F.S.C. reveals results of three kinds, blows, strikes and abortive blows. The first of these is represented by a compact mass of from 100-300 eggs situated well down in the thickness of the fleece, this being the site in which *Lucilia sericata* will deposit its eggs under natural conditions. A strike is represented by a cluster of larvae actively feeding on the skin and, as has already been explained, is for the purposes of this test treated as having the same significance as a blow. An abortive blow consists of a few scattered eggs, or a small cluster of not more than 20 eggs, situated high up near the tips of the fleece. Their presence can, perhaps, best be explained by a consideration of the natural habits of *L. sericata*.

Observations in the F.S.C. on the act of oviposition by the gravid fly have shown that there is always a careful selection of a suitable site prior to egg-laying. This process, in nature, involves in the first place a selection of the actual part of the sheep to be blown; under our conditions where a potent "attractant" is used, the fly invariably alights in the vicinity of this "attractant", and she spends a considerable time (3 to 10 mins.) making a closer examination of the site, during which time she creeps in and out of the fleece. Finally if conditions are satisfactory, she settles down some distance from the tips of the fleece, extrudes her ovipositor deeper still, and proceeds to deposit her full complement of eggs in a single cluster.

It will be clear then, that the presence of a small cluster of eggs (less than 20) high up near the tips of the fleece does not constitute a natural deposition, and further, in this position the eggs lack the necessary conditions (constant high humidity, etc.) for development. These observations are in agreement with those of other workers such as Maldwyn Davies and Hobson (1935) who showed that the eggs must be deposited deep down in the fleece and near the skin, where a natural high humidity is maintained, and without which development is impossible. Thus these small, abortive, ill-placed blows cannot be regarded as potential strikes, and may therefore be ignored in assessing the breaking point of a dip.

Abortive blows on the other hand may be of significance in indicating the presence of an insecticide. When DDT is present in the fleece, it has been observed that the gravid fly will spend almost as much time probing the prospective site for egg-laying, as she does on an untreated sheep. In a short time (3 to 5 mins.) toxic symptoms become apparent, yet even then the fly often persists in her probing operation, and may not leave the sheep until almost total paralysis of the legs has taken place. Flies in this condition, almost moribund, have been closely observed and seen to extrude a small number of eggs apparently as an involuntary act. It is thought therefore, that abortive blows may be the result of such a process, and if so, only serve to indicate the presence of an active insecticide.

Application of results to assessment of protective values.

There are several ways in which the results obtained from the "anti-adult" and larvicide tests, can be used to assess the protective value of the agent concerned. The incidence respectively of blows, or strikes, occurring in one group during a certain period, can be compared with that for another treated group, or the control group, during the same period. Alternatively the time at which the first sheep in each group is blown or struck may be taken as the significant point. Both these methods are, however, open to criticism. In spite of the most careful selection of sheep, and the levelling effect of the "attractant", a number of very susceptible individuals will still exist. It has frequently been observed that a single sheep will be blown at an

early date and will continue to be blown in subsequent weeks, yet it is not until much later that the remainder of the sheep in the group show any signs of a break in protection. Such a susceptible sheep should not be allowed to bias the results.

A more reliable method of assessing results is to consider the group as a whole, and to use, as an index, the time at which a certain proportion of the group has failed. In this way a susceptible individual which is blown unduly early is not considered again, and in order to establish any further break in the group a second individual must be blown. In using this method it is necessary to select an arbitrary point, considered of maximum significance, when the protection is deemed to have failed for the group as a whole. For the purposes of the present work, where groups of five or six sheep are used, the point which has been selected is that at which the second sheep in the group is blown or struck, provided that a third sheep becomes affected within a reasonably short time. It will be seen, therefore, that approximately a 50 per cent. breakdown of the group is taken as the significant point, and that in this way any error due to an unusually susceptible, or immune, sheep is eliminated. This method of assessing results is considered to be a fair way of *comparing* the protective properties of the several agents under test; it is not suitable for estimating a practical period of protection, since in no field trial would a 50 per cent. breakdown in a flock be used as the criterion of the failure of a maggot fly dip.

Experimental Data.

During the summer and autumn of 1947 and 1948, the two techniques described were employed to test the protective properties of a number of agents used for maggot fly control; in addition, the comparative values of different methods of application were investigated. In this part of the paper three series of results are presented, which serve to illustrate the working of the techniques, and provide some information regarding the protective properties of the agents tested.

As a result of early work in the F.S.C., when DDT dipped sheep were being tested against undipped controls, it was possible to confirm the view expressed by Cragg (1946), namely that DDT possesses no marked larvicidal action, and that it exerts its effect primarily against the adult fly. A study of the development rate of blows to strikes under F.S.C. conditions, revealed the fact that there was no significant difference in this rate on DDT dipped sheep as against that on concurrent controls; DDT cannot, therefore, have interfered with larval development. It follows then, that in testing preparations containing DDT alone, it is not necessary to employ the larvicide test.

In the first two series of results given here DDT preparations only are included, and therefore the F.S.C. test is adequate. In the third series of results, DDT is tested against Arsenic and BHC; since these possess larvicidal properties, the full technique is necessary, *i.e.*, the F.S.C. "anti-adult" test followed by the larvicide test.

The DDT used in these experiments was technical 1:1:1-trichloro-2:2-bis(*p*-chlorophenyl)ethane containing 80 per cent. of the *para para'* isomer. The BHC was technical 1:2:3:4:5:6-hexa-chlorocyclohexane containing 13-15 per cent. gamma isomer.

SERIES I.

Test of DDT treated sheep versus controls.

Preparations.

- | | | | | |
|-----|---|-----|-----|----------|
| (1) | DDT water suspension (DDT 0.5 per cent.) | ... | ... | Dipped |
| (2) | DDT water suspension (DDT 0.25 per cent.) | ... | ... | Dipped |
| (3) | DDT water suspension (DDT 0.5 per cent.) | ... | ... | Sprayed |
| (4) | DDT in Benzene Emulsion (DDT 0.5 per cent.) | ... | ... | Dipped |
| (5) | DDT dry powder (DDT 5.0 per cent.) | ... | ... | Powdered |

Mode of Application.

Dipping was carried out in a 240-gallon bath, each sheep receiving a half minute immersion. The sheep were in "short fleece" and carrying six weeks' growth of wool at the time of treatment.

The spray was applied by means of a bucket pump fitted with a spray nozzle. Half a gallon of wash was distributed as evenly as possible over the entire body of the sheep.

The powder was shaken on to the fleece from a sprinkler-topped canister and rubbed in well by hand. Approximately half a pound of powder was used to dress the entire sheep.

The weekly results obtained from testing this series of preparations in the F.S.C. are recorded in Tables I and II.

TABLE I.
Weekly results in the Fly-Sheep Chamber.

Preparation	Group No.	Sheep No.	Weeks after treatment									
			1	2	3	4	5	6	7	8	9	10
(1) 0.5% DDT Suspension	I Dipped	1	—	—	—	—	—	—	—	—	—	—
		2	—	—	—	—	—	—	—	—	—	—
		3	—	—	S	B	B	S	S	+	—	—
		4	—	—	—	—	—	—	—	B	S	—
		5	—	—	—	—	—	—	—	—	S	S
(2) 0.25% DDT Suspension	II Dipped	6	—	—	—	—	—	—	—	—	—	—
		7	—	B	—	B	—	S	S	+	—	—
		8	—	—	—	—	—	—	—	—	—	—
		9	—	—	—	—	—	B	S	—	—	—
		10	—	—	—	—	—	—	—	—	B	S
(3) 0.5% DDT Suspension	III Sprayed	11	—	—	—	—	S	S	—	—	—	—
		12	—	—	—	—	B	S	—	—	—	—
		13	—	—	—	—	—	—	—	—	—	—
		14	—	B	—	—	B	B	—	—	—	—
		15	—	—	—	—	—	—	—	—	—	—
(4) 0.5% DDT Emulsion	IV Dipped	16	—	—	—	—	S	S	—	—	—	—
		17	—	—	—	—	—	—	—	—	—	—
		18	—	—	—	—	S	—	—	—	—	—
		19	—	—	—	B	B	—	—	—	—	—
		20	—	—	—	—	—	—	—	—	—	—
(5) 5.0% DDT Dry Powder	V Powdered	21	—	—	—	B	—	—	—	—	—	—
		22	—	—	—	—	S	—	—	—	—	—
		23	—	—	—	B	S	—	—	—	—	—
		24	—	S	—	—	S	—	—	—	—	—
		25	—	—	—	—	S	—	—	—	—	—
Controls	10 sheep		S	S	BS	BS	BS	BS	BS	BS	B	S
				S	BS	BS	BS	BS	BS	BS	B	S
					BS	BS	BS	BS	BS	BS		S
						B	B	B	S	S		S
						B	B			S		S

B = Blow.

S = Strike.

* = Only five controls were exposed.

+ = This sheep was eliminated here.

The results given in Table I are presented again in Table II, in order to illustrate what proportions of sheep in each group had been blown or struck up to any given time.

TABLE II

Preparation	Group No.	Sheep No.	Weeks after treatment									
			1	2	3	4	5	6	7	8	9	10
(1) 0.5% DDT Suspension	I Dipped	1	○	○	○	○	○	○	○	○	○	○
		2	○	○	○	○	○	○	○	○	○	○
		5	○	○	○	○	○	○	○	○	●	●
		4	○	○	○	○	○	○	○	●	●	●
		3	○	○	●	●	●	●	●	●	●	●
(2) 0.25% DDT Suspension	II Dipped	6	○	○	○	○	○	○	○	○	○	○
		8	○	○	○	○	○	○	○	○	○	○
		10	○	○	○	○	○	○	○	○	●	●
		9	○	○	○	○	○	●	●	●	●	●
		7	○	●	●	●	●	●	●	●	●	●
(3) 0.5% DDT Suspension	III Sprayed	12	○	○	○	○	○	○				
		11	○	○	○	○	●	●				
		13	○	○	○	○	○	○				
		15	○	○	○	○	●	●				
		14	○	●	●	●	●	●				
(4) 0.5% DDT Emulsion	IV Sprayed	19	○	○	○	○	○	○				
		17	○	○	○	○	○	●				
		16	○	○	○	○	○	●				
		18	○	○	○	○	●	●				
		20	○	○	○	●	●	●				
(5) 5.0% DDT Dry Powder	V Powdered	22	○	○	○	○	●					
		25	○	○	○	○	●					
		21	○	○	○	○	●					
		23	○	○	○	○	●					
		24	○	●	●	●	●					

Each group consists of five sheep which were exposed in the F.S.C. at weekly intervals. Blank circles indicate weekly exposures of individual sheep. When a break in protection (blow or strike) occurs on any sheep that fact is indicated by a blacked-out circle. The same symbol is retained to indicate subsequent exposures whether or not this sheep is again blown or struck.

SERIES II.

The effect of fleece length on protection.

Preparations.

(1) DDT water suspension (DDT 0.5 per cent.)	Long fleece	Dipped
(2) DDT water suspension (DDT 0.5 per cent.)	Short fleece	Dipped
(3) DDT white spirit emulsion (DDT 0.25 per cent.)	Long fleece	Sprayed
(4) DDT white spirit emulsion (DDT 0.25 per cent.)	Short fleece	Sprayed

Dipping and spraying was carried out on the respective groups in the same way as that already described for Series I.

The sheep in "short fleece" were carrying six weeks' growth of wool at the time of treatment. Those in "long fleece" were unshorn and were therefore carrying a full 12 months' growth of wool.

For the sake of clarity, the detailed weekly results have been omitted, and the proportional breakdown of the groups is presented directly in Table III in the same form as that employed in Table II.

TABLE III.

Preparation	Group No.	Weeks after treatment												
		1	2	3	4	5	6	7	8	9	10	11	12	13
(1) 0.5% DDT Water Suspension	I				○	○	○	○	○	○	○	○		
	Dipped Long fleece				○	○	○	○	○	○	○	○		
(2) 0.5% DDT Water Suspension	II			○	○	○	○	○	○	○	○	○	○	○
	Dipped Short fleece			○	○	○	○	○	○	○	○	○	○	○
(3) 0.25% DDT Emulsion	III			○										
	Sprayed Long fleece			○										
(4) 0.25% DDT Emulsion	IV			○	○	○	○	○	○					
	Sprayed Short fleece			○	○	○	○	○	○					

SERIES III.

Test of DDT, BHC and Arsenic dips.

Preparations.

- | | | | | |
|-----|--|-----|-----|--------|
| (1) | DDT water suspension (DDT 0.5 per cent.) | ... | ... | Dipped |
| (2) | BHC water suspension (BHC 0.125 per cent.) | ... | ... | Dipped |
| (3) | Arsenic-sulphur (Proprietary) (As_2O_3 0.2 per cent.) | ... | ... | Dipped |

These preparations were applied to the sheep by dipping in a 240 gallon bath, with an immersion time of half a minute.

In testing the above preparations it is necessary to employ the larvicide test in addition to the F.S.C. technique, since Arsenic is known to exert its effect primarily as a larvicide, whilst the mode of action of BHC was at that time less certain. The routine therefore involves the exposure of all groups in the F.S.C. until a break in any group occurs (Table IV), this group is then subjected to the larvicide test in subsequent weeks until larvicidal protection is also shown to have failed (Table V).

TABLE IV.
F.S.C. Results or "Anti-adult" Test.

Preparation	Group No.	Weeks after treatment												
		1	2	3	4	5	6	7	8	9	10	11	12	13
(1) DDT Suspension	I Dipped			○	○	○	○	○	○	○	○	○	○	○
				○	○	○	○	○	○	○	○	○	○	○
				○	○	○	○	○	○	○	○	○	○	○
				○	○	○	○	○	○	○	○	○	○	○
				○	○	○	○	○	○	○	○	○	○	○
(2) BHC Suspension	II Dipped		○	●										
			●	●										
			●	●										
			●	●										
			●	●										
(3) Arsenic-sulphur	III Dipped		○	●										
			○	●										
			○	●										
			○	●										
			○	●										

TABLE V.
Larval Implantation Results or Larvicide Test.

Preparation	Group No.	Weeks after treatment												
		1	2	3	4	5	6	7	8	9	10	11	12	13
(1) DDT Suspension	I Dipped												●	
													●	
													●	
													●	
													●	
(2) BHC Suspension	II Dipped		○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
(3) Arsenic-sulphur	III Dipped		○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
			○	○	○	○	○	○	○	○				
Controls						18		4	3	4	*			
						20		4	4	5				

* The upper figure indicates the number of controls which developed strikes. The lower figure indicates the total number of controls implanted on each occasion. Where no figure is given no controls were tested.

Discussion and Conclusions.

Before proceeding to a consideration of the foregoing results, it is necessary to clarify one point in connection with comparisons made between results in different series. It will be observed that a DDT suspension dip, at a concentration of 0.5 per cent., has been tested in Series I and II on sheep carrying the standard six weeks' growth of wool. The resulting protection is not found to be the same in both tests, being approximately eight weeks in Series I and twelve weeks in Series II. This type of discrepancy is found to occur between different tests, and is to be explained by the very different climatic conditions to which the sheep may be exposed during the course of two separate tests. For example, Series I was tested during the hot dry summer of 1947, and Series II during the wet summer of 1948; it is to be expected that a very different weathering of the fleece took place on each occasion. Nevertheless within one series, all groups of sheep are exposed to identical conditions and therefore, comparisons within a series are justified, whilst those between different series are not valid.

An exception to this rule is permissible with regard to Series II and III since all these groups were, in fact, tested at the same time, and are here separated into two series only in order to present the results more clearly. It will be observed that the same DDT group is included in each series, and this has been done in order to provide a standard basis for comparison with other agents. A 0.5 per cent. DDT suspension dip has, in the past, been widely tested in field trials and the inclusion of this agent in all "anti-adult" and larvicide tests, provides a valuable means of relating the present results to those which might be expected under field conditions.

Interpretation of Results. Series I.

By making use of the method already described of assessing a significant breaking point, namely the time at which the second sheep in a group fails provided this is followed reasonably soon by failure of the third sheep, the following results are obtained from Table II.

	<i>Preparation</i>	<i>Failure of Protection</i>
	DDT suspension dip (DDT 0.5 per cent.)	8 weeks
	DDT suspension dip (DDT 0.25 per cent.)	6-7 weeks
	DDT suspension spray (DDT 0.5 per cent.)	4-5 weeks
	DDT emulsion dip (DDT 0.5 per cent.)	4-5 weeks
	DDT dry powder (DDT 5.0 per cent.)	3-4 weeks

In drawing conclusions from these results, it must be re-emphasised that the above figures must not be taken literally as the periods of protection which will obtain under field conditions, but only as a basis of comparison for the agents tested in this series.

Viewed in this light, it is clear that the DDT suspension is the best formulation of the insecticide in the present series, and that dipping is a more efficient method of application than spraying when the latter is carried out at the rate of half a gallon per sheep. The figure of six to seven weeks as against eight weeks, indicates that the period of protection is not seriously affected when the concentration of DDT in a dip is reduced from 0.5 per cent. to 0.25 per cent. In terms of practical application, however, it might be unwise to reduce the concentration to the lower figure, since in the preparation of a dip due allowance must be made for the exhaustion of the wash which takes place when a large number of sheep are passed through the bath. In the experiments described here, the whole question of exhaustion has purposely been ignored, and a fresh bath was always prepared for dipping each of the small experimental groups.

The protection provided by the DDT benzene emulsion dip is markedly inferior to that given by the suspension type dip whether the latter be used at the same or a lower concentration, whilst the application of 5 per cent. DDT powder is also shown to be unsatisfactory.

In considering agents with poor protective properties, there is a further factor which must be taken into account under F.S.C. conditions. In the same way that control sheep in the field gain some measure of protection by grazing near DDT treated sheep, so sheep with a poor protective dressing are to some extent protected in the F.S.C. by being exposed in company with sheep carrying a good dressing. This effect is seen in the low incidence of blow or strike recorded on the control sheep (Table I) during the first one or two weeks of the test. It is probable, therefore, that the efficiency of a dressing such as the 5 per cent. DDT dry powder is even less than is indicated in the present test. This "interference factor" can be eliminated by exposing each group of sheep separately, but it is felt that this method detracts from the exact comparative nature of the test.

Interpretation of Results. Series II (Table III).

Preparation		Application		Fleece	Failure of protection
DDT suspension	...	Dipped	{	Long	10 weeks
DDT 0.5 per cent.	...			Short	12 weeks
DDT emulsion	...	Sprayed	{	Long	less than 3 weeks
DDT 0.25 per cent.	...			Short	6 weeks

Here the effect of fleece length on the protection provided by a dip and a spray is investigated. It is unfortunately not possible to make any direct comparison between the dipped group and the sprayed group owing to the different DDT concentration and formulation in each group. It might be observed, however, that in Series I a 0.25 per cent. suspension dip was found to be only slightly inferior to the same dip at 0.5 per cent. concentration. In this series it seems improbable, therefore, that the lower DDT concentration in the sprayed group is responsible for the inferior protection, and that this is due rather to the formulation as an emulsion, or to spraying as a method of applying the insecticide. Such a view is in agreement with the results obtained in Series I.

Turning to the effect of fleece length in the dipped groups, it is rather surprising to find that the sheep in full fleece received a slightly shorter protection than those in short fleece. One would expect that the long woolled sheep, dipped in the same way as the short, would have a thicker and more resistant barrier of DDT impregnated wool, which would in turn provide a more efficient and lasting protection. However, observations on these sheep at the time the dip was breaking provide a reasonable explanation.

It is known that the maggot fly spends a considerable time on the sheep prior to oviposition, and that during this time she creeps in and out of the fleece selecting a suitable site. In the presence of active DDT she collects a sublethal dose of the insecticide which acts sufficiently quickly to produce symptoms of restlessness and even excitement, and she will leave the sheep without depositing eggs. When a dip is on the point of breaking, the above symptoms are exhibited more slowly but are still sufficient to encourage the fly to leave the sheep if she can. On long woolled sheep, however, the fly is often quite deeply buried in the wool and it frequently happens that in her slightly intoxicated condition she becomes entangled in the wool

fibres and is unable to escape. During her struggles a fly will often, perhaps involuntarily, expel a number of eggs which, when the insecticide is becoming slower in action, may amount to a considerable number. Such blows differ from the natural type in being scattered and ill-placed, but they are sufficiently large to represent a potential strike and cannot therefore be discounted. It is blows of this type, usually lying round about the dead fly, which were encountered when the long woolled group was breaking, and the fact emerges that the premature breakdown of this group was due to mechanical effects, rather than to any fundamental difference in the action of DDT when applied to long or short fleeced sheep.

The difference in the protection conferred on sheep with six weeks' growth of fleece as opposed to those in full fleece, when sprayed with a DDT emulsion, is most marked. The quantity of spray used was half a gallon per sheep, and this was applied as evenly as possible to the entire body area. On sheep carrying a short fleece the coverage is fairly adequate but on sheep in full fleece the application of this quantity does little more than wet the tips of the fleece. Subsequently the fly has no difficulty in penetrating the thin DDT impregnated barrier and gaining the comparative safety of the clean wool beneath, in which she is completely free to lay her eggs at leisure. Alternatively, with sheep in full fleece, partings frequently appear in the wool as the sheep moves; these expose the clean underlying wool and the fly is able to alight directly on to this area and lay her eggs, without ever coming into contact with the more superficial layer carrying the insecticide.

These observations, when viewed from the practical aspect, raise some doubts as to the value of spraying unshorn sheep early in the maggot fly season. It is true that the application of a spray to such sheep is often the only practicable method of control prior to shearing, but it is clear that the degree of control obtained in this way is likely to be unreliable, unless a quantity of spray considerably in excess of half a gallon is applied to each sheep.

Interpretation of Results. Series III (Tables IV and V).

In order to assess the comparative protective values of the agents included in this series, it is first necessary to establish the period of "anti-adult" protection from Table IV. A study of Table V will now indicate what larvicidal protection, if any, remains after the "anti-adult" protection has failed. It will be appreciated that no test of larvicidal properties is carried out until the "anti-adult" protection has failed, since no larvae can be present whilst the sheep are still efficiently protected against blow.

Preparation	Failure of "Anti-adult" protection	Failure of Larvicidal protection
DDT suspension dip DDT 0.5 per cent.	12 weeks	less than 12 weeks
BHC suspension dip BHC 0.125 per cent.	less than 2 weeks	7-8 weeks
Arsenic-sulphur dip As ₂ O ₃ 0.2 per cent.	less than 2 weeks	6-8 weeks

The chief point of interest in these results lies in the fact that they demonstrate clearly the very different mode of action of DDT as opposed to BHC and arsenic. It will be observed that the protection provided by DDT is due purely to a persistent "anti-adult" action and that, when at the end of 12 weeks this has failed, there is no residual larvicidal effect. Whether or not DDT is effective as a larvicide nearer the

time of dipping is not demonstrated by these results but, so far as protection is concerned, the question is irrelevant so long as its "anti-adult" action is efficient. Other results quoted earlier in this paper have, however, shown that any larvicidal properties which DDT may possess are of very minor significance.

BHC and arsenic, on the other hand, possess virtually no "anti-adult" action at the above concentrations, this being demonstrated by their complete breakdown on the first occasion when they were exposed in the F.S.C. during the second week. When, however, the larvicide test was applied in the following week, it was found that the development of strike was prevented, and it was not until much later that the larvicidal effect was found to fail.

It is unfortunately not possible to make a direct comparison between the values of DDT and BHC in this series, owing to the fact that BHC was tested at a concentration of only 0.125 per cent. This is the strength at which BHC is normally used for the control of Sheep Scab. This series was tested primarily in order to determine the comparative merits of arsenic and BHC against the sheep maggot fly, as an incidental effect when they were used for their basic purpose of scab control. The test has indicated that there is likely to be little significant difference between them so far as maggot fly control is concerned.

Some criticism might perhaps be levelled at the larvicide test in that, on this occasion, it assigns a protection of six to eight weeks to arsenic, when it is known that under field conditions the figure is in the region of only three to four weeks. The danger of accepting results from these tests as having anything more than comparative value has already been stressed, and it is felt that this limitation is of even greater importance in connection with the larvicide test. It seems probable that climatic variations of humidity and temperature do to some extent vary the severity of the test, and that this variation will not be reflected in the rate of development of strikes on control sheep, where a success of over 75 per cent. obtains under all conditions. Such climatic changes, within normal limits, have little if any effect on the ovipositing adult fly, and it may therefore be unwise to make direct comparisons between "anti-adult" test results on the one hand and larvicide test results on the other. A better method of assessment is to employ an agent such as 0.5 per cent. DDT, of known "anti-adult" efficiency, as a standard in the one test, and a different agent such as arsenic of known larvicidal efficiency, as the standard in the other test. Extensive field trials have shown that the protection provided by a 0.5 per cent. DDT suspension dip is approximately seven weeks, whilst a normal arsenic-sulphur dip fails at from three to four weeks. By making use of these two agents as indices it is possible to establish the probable value which other agents will possess when tested under field conditions.

Summary.

A controlled technique is described, whereby the comparative protective values of various dips and sprays used in sheep maggot fly control can be accurately assessed.

In order to determine the exact mode of action of the agents under test, each is examined from the point of view of its power to inhibit oviposition by the adult fly and also for its larvicidal properties.

The "anti-adult" test involves the weekly exposure of treated groups of sheep to an active and dense fly population, maintained in a closed chamber under controlled conditions of temperature and light.

The larvicide test is a modification of a former method of larval implantation, and is applied to the sheep when the "anti-adult" protection, indicated by the previous test, is found to have failed.

In the second part of the paper, a number of results are presented which serve to illustrate the working of the technique, and demonstrate the comparative value of several methods of maggot fly control which are in current use.

A dip containing 0.5 per cent. DDT in water suspension was found to give the most satisfactory protection, and to achieve this result purely by its action against the adult fly. A 0.5 per cent. DDT benzene emulsion dip was less satisfactory, as also was spraying as a method of applying the insecticide. In general, spraying has given about half the protection of dipping, whilst on sheep in full fleece, the application of half a gallon of spray was found to be inadequate. Protection was again found to be poor when the fleece was carefully dressed with half a pound of a dry powder containing 5 per cent. DDT.

In contrast to DDT, BHC and arsenic were shown to possess virtually no "anti-adult" properties at the concentrations tested. BHC and arsenic both acted as efficient larvicides and provided a valuable and similar degree of protection against the sheep maggot fly, as an incidental effect, when they were tested at the concentrations normally used for their primary function of sheep scab control.

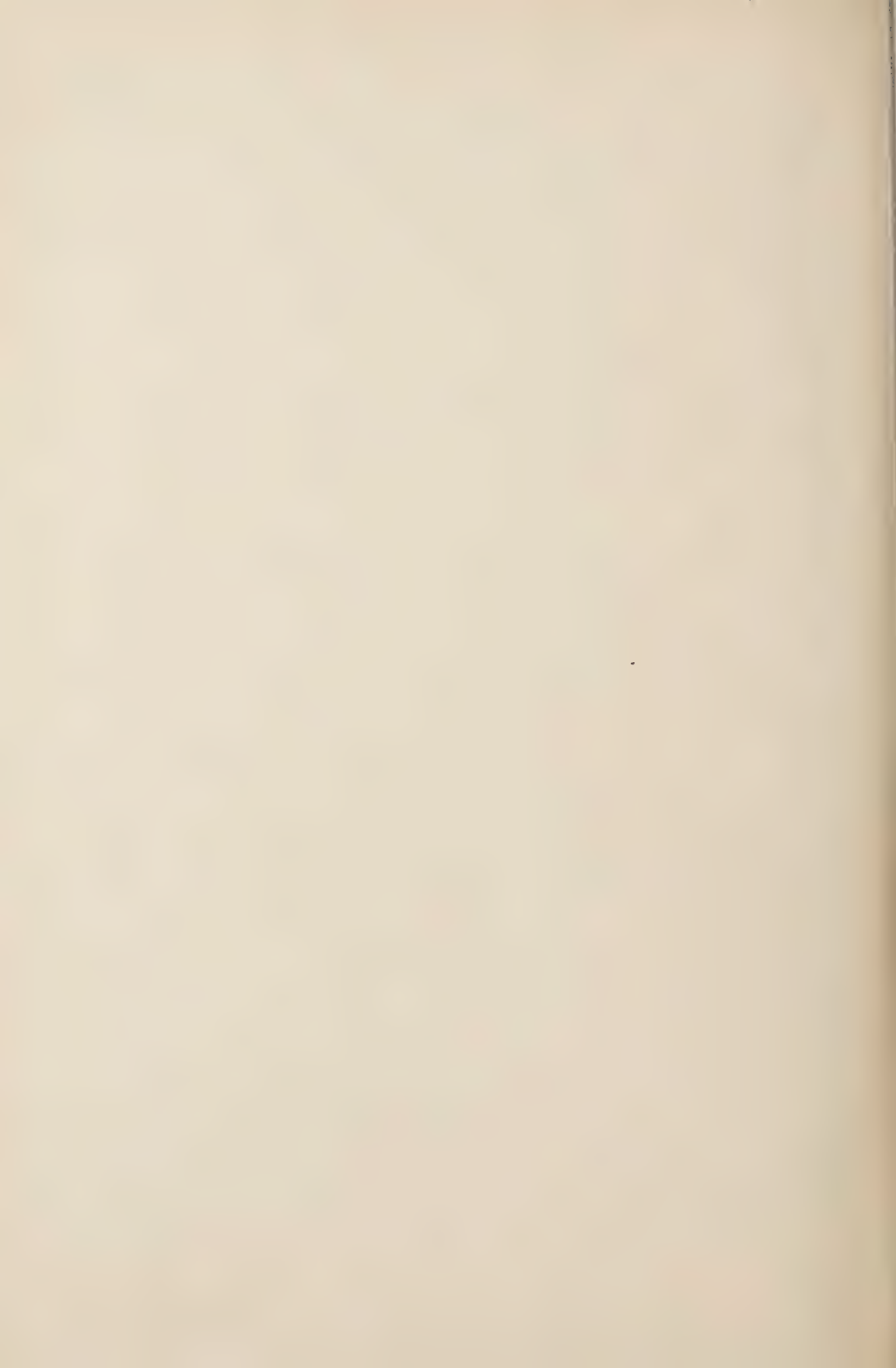
Acknowledgements.

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References.

- Cragg, J. B. (1946). *Ann. appl. Biol.*, **33**, pp. 127-129.
Davies, W. M. & Hobson, R. P. (1935). *Ann. appl. Biol.*, **22**, pp. 279-293.
Freney, M. R., MacKerras, I. M., & MacKerras, M. J. (1935). *J. Council. sci. industr. Res. Aust.*, **8**, pp. 161-168.
Hobson, R. P. (1935). *Ann. appl. Biol.*, **22**, pp. 294-300.
Hobson, R. P. (1936). *Ann. appl. Biol.*, **23**, pp. 845-851.
Hughes, L. E., Jenkins, J. R. W. & Jones, J. M. (1946). *Vet. Rec.*, **58**, pp. 251-252.
MacLeod, J. (1937). *Parasitology*, **29**, pp. 526-529.
MacLeod, J. (1933). *Bull. ent. Res.*, **29**, pp. 149-163.
Stamp, J. T., Watt, J. A. & Beattie, I. S. (1948). *Vet. Rec.*, **60**, pp. 335-336.
Tillyard, R. J. & Seddon, H. R. (1933). *Pamphl. Coun. sci. industr. Res. Aust.* no. 37, pp. 126-128.
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ILLUSTRATIONS OF TSETSE LARVAE.

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Some of the structures illustrated here have already been noticed in two previous papers (Jackson, 1948a & b). All the present drawings were made by one of us (E.B.) from living material of *Glossina swynnertoni* Austen. The eggs and the first- and second-instar larvae were dissected from wild female flies and examined in saline under the microscope; the third-instar larvae were obtained by keeping females in captivity until larviposition occurred, normally in the late afternoon.

The ovulated egg is illustrated in lateral outline in fig. 1. It measures about 1.5 mm. in length and is broader towards the hind end, concave dorsally and convex ventrally. The anterior integument is differentiated into a more translucent micro-pylar region; the remainder of the surface of the chorion is sculptured in a diamond pattern (fig. 2), which under higher magnification is seen to be composed of minute, wart-like excrescences arranged in rows. Details of the freshly dissected egg seen by



Fig. 1.—Outline of egg from left side ($\times 33$).

Fig. 2.—Egg viewed from dorsal aspect by transmitted light ($\times 66$).

Fig. 3.—Newly hatched first-instar larva from left side ($\times 33$). Note tracheal trunk and oral egg-tooth projecting above.

transmitted light are at first entirely obscure, but when left for a few minutes in saline the contents shrink and the structure of the shell outside them can be seen. Parts of the shell viewed by transmitted light are usually, but not always, black and opaque because of a white waxy deposit. This is indicated in fig. 2 by the black tongues obscuring the pattern and following its sculpture; the remaining black part depicts the dense and opaque contents of the egg.

Fig. 3 is a diagrammatic drawing of the newly hatched first-instar larva seen from the left side. The respiratory lobes of the later instars are here undeveloped; the most conspicuous structures are the paired tracheal trunks and their branches, seen from the dorsal side in fig. 4. The mouth occupies an antero-dorsal position and is blocked by a movable chitinous egg-tooth (figs. 3 & 4) used to puncture the chorion, which then splits on a line of weakness along the dorsal side.

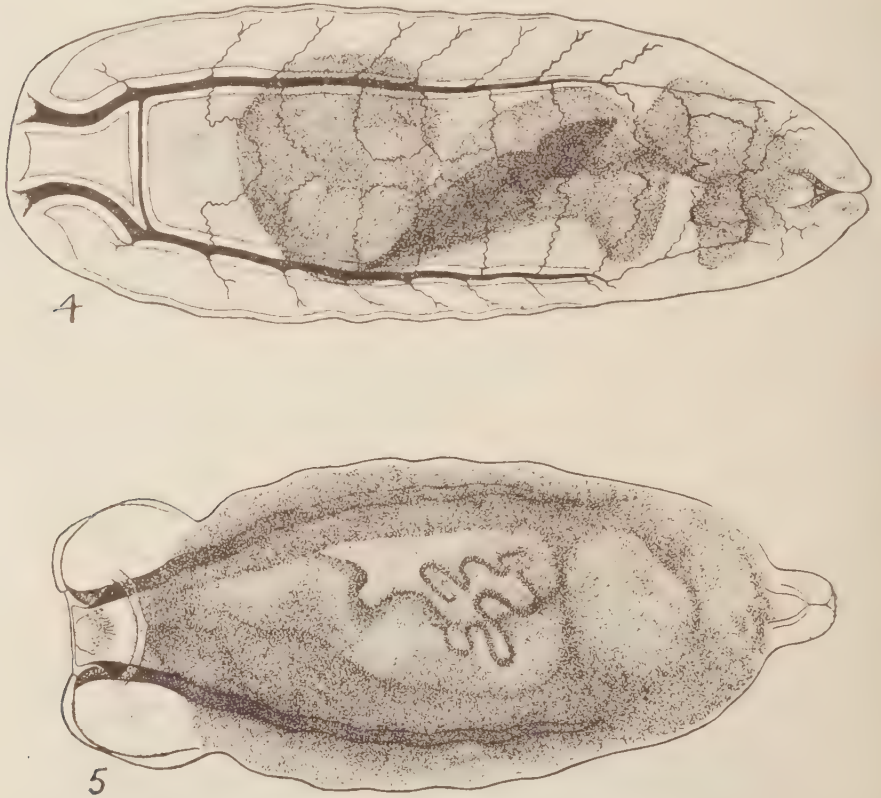


Fig. 4.—First-instar larva from dorsal aspect ($\times 66$). Note tracheal trunks and branches, and egg-tooth in mouth.

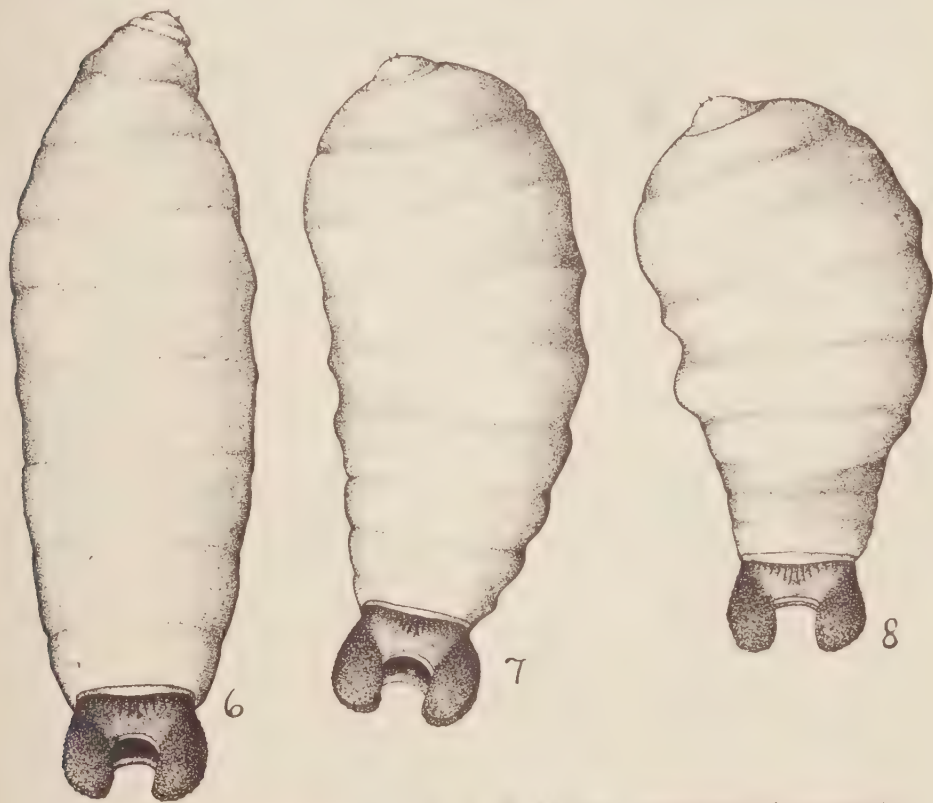
Fig. 5.—Second-instar larva from dorsal aspect ($\times 26$).

The first-instar larva is apparently incapable of feeding, and the oral egg-tooth prevents attachment to the teat of the milk glands of the mother. According to Roubaud (1909) it moults after only a few hours. The "first instar" larva of *G. palpalis* (R.D.) depicted by Newstead, Evans & Potts (1924, Pl. II, fig. 1) is an early second-instar. Roubaud (1909) gives a diagrammatic drawing (fig. 101) of the first-instar larva of *G. palpalis*.

The second instar (fig. 5) has partly developed respiratory lobes, and between them are rows of bristles up to 12 microns in length. The general integument, like that of the first instar, is clothed with short spines from 1 to 3 microns long. The chitinated antenno-maxillary complex of the third instar is undeveloped. The second-instar moults on attaining a length of about 4 mm.

The third instar, growing to a length of about 7 mm., has well-developed respiratory lobes, which, according to our colleague Mr. W. H. Potts, blacken about one and a half days before larviposition. At about the same time the antenno-maxillary appendages and the functionless anus also blacken.

Figs. 6 to 11 illustrate the mode of progression of the freshly deposited larva. They are drawn from a ciné film taken by Mr. C. J. Webb with a camera kindly lent by Dr. P. E. Glover.

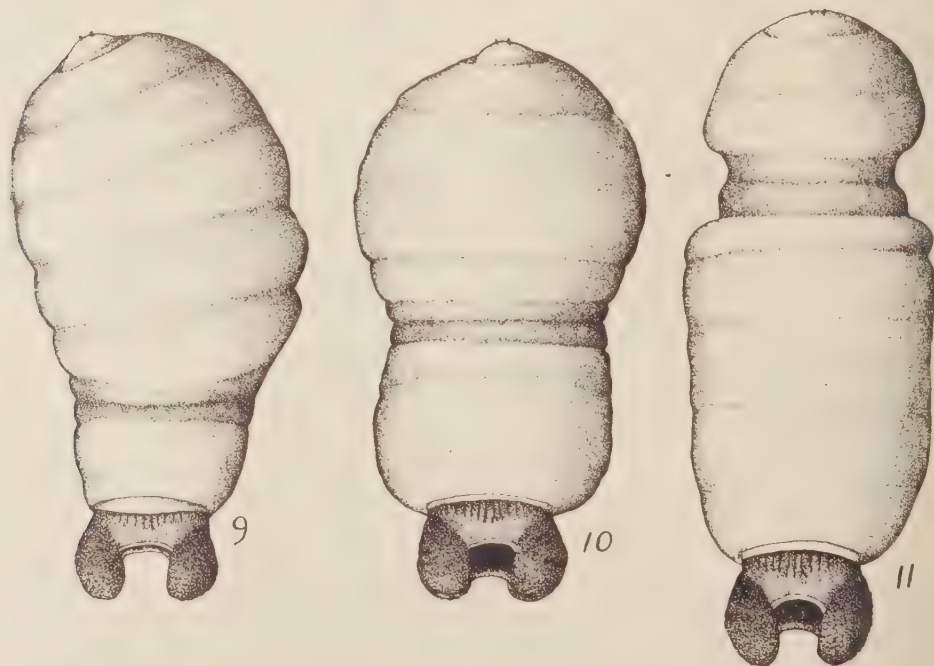


Figs. 6-8.—Third-instar larva. All $\times 14$. (6) At 0 sec., completion of movement ; (7) At $\frac{1}{4}$ sec., retracting head ; (8) At $\frac{3}{8}$ sec., with respiratory lobes and hind end drawn forward, and lobes laid flat.

Fig. 6 shows the larva in the fully extended position, at the end of the forward movement, with the head characteristically questing from side to side before the next movement is begun. The posterior respiratory lobes are directed somewhat dorsally, so that the cavity between them is visible from above. In fig. 7 ($\frac{1}{4}$ sec. later), the larva has retracted the previously extended head preparatory to making its forward movement. At $\frac{3}{8}$ sec. the posterior end is drawn forward and the body is arched, recalling a Geometrid larva, and the respiratory lobes are then laid flat (fig. 8). A wave of contraction now ($\frac{1}{2}$ sec.) appears near the posterior end and begins to travel forward (fig. 9), forcing the contents of the larva forward in front of it. In fig. 10 ($\frac{3}{4}$ sec.) this wave of contraction has advanced to the middle of the larva and in fig. 11 (1 sec.) it is nearing the anterior end. Finally after $1\frac{3}{8}$ sec. the condition is similar to that shown in fig. 6, with the larva fully extended and the mobile head

exploring sideways before the next movement is begun. Some of these motions are shown diagrammatically by Roubaud (1909, fig. 99) ; we personally found that the motions of the larva were just too fast to permit a correct interpretation of their sequences without the aid of the ciné film (16 exposures per second).

In drawing the third-instar larva we came upon an unexpected difficulty in representing its segmentation. Roubaud (1909) says that the larva has 12 segments (3 thoracic and 9 abdominal ; the cephalic segment is not externally visible as such), but from his description it is clear that he regards the spiracles themselves as the 9th abdominal segment. Newstead, Evans & Potts (1924) give the same number of segments (3 thoracic and 9 abdominal), but they are not the same as those of Roubaud, for they treat the spiracles, correctly as we believe, as protuberances of the 8th abdominal segment, and their 9th segment is that which bears the anus ; it is described as being displaced ventrally, and is invisible on the dorsal side of the larva. We do not agree with either interpretation ; our conclusions are based on examinations of larvae made by each of us separately, and we have independently concluded that not only one, but two segments are "displaced ventrally". Thus we believe, with Newstead, Evans & Potts, that the 8th abdominal segment bears the spiracles ; but that it is the 10th and not the 9th segment which bears the anus. The 9th segment, like the 10th, is "displaced ventrally", and appears on the ventral side as a broad band between the 7th and 10th segments, but does not reach the



Figs. 9-11.—Third-instar larva. All $\times 14$. (9) At $\frac{1}{4}$ sec., with wave of contraction starting near hind end ; (10) At $\frac{1}{2}$ sec., with wave of contraction advanced to half-way ; (11) At 1 sec., with wave of contraction nearly arrived at front end.

middle line on the dorsal side (fig. 12). It would on our interpretation be more correct to say that, having been displaced dorsally, the 8th segment has overgrown the 9th and 10th on their dorsal sides, and has overlapped them behind rather than to describe the 9th and 10th segments as "displaced ventrally". If so, the 8th

abdominal segment has been pinched out on the ventral side and its topographically ventral portion, between the anal segment and the respiratory lobes, is morphologically dorsal. It might thus be expected that the hind gut would curve forward from the anus through the 9th segment, then backwards to the 8th and forwards to the region of the 7th segment. Examination of sections of a first-instar larva showed that there is in fact a slight backward curve into the 8th segment; the apparent absence of any forward curve into the 9th might be explained by the very small size of the reduced 10th segment.

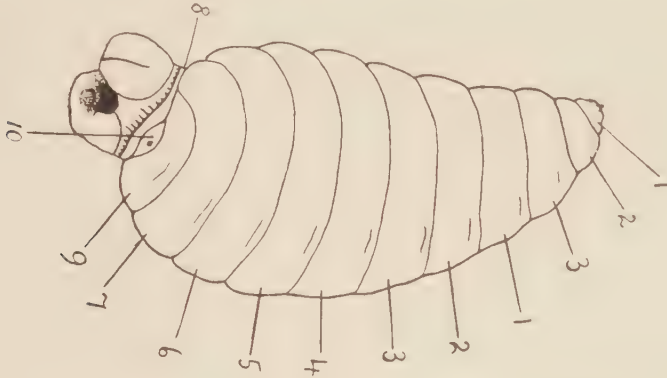


Fig. 12.—Third-instar larva from ventro-lateral aspect. Diagrammatic drawing showing segmentation ($\times 14$).

Roubaud (1909, fig. 100 and Pl. 2, fig. 6) shows the anus in the wrong position, close to the base of the respiratory lobes. Thinking that this might be because he was dealing with *G. palpalis* while we were examining *G. saynvertoni*, we have since looked at both larvae and pupae of *G. palpalis*, but still find that the anus is where we have shown it (fig. 12), near to the posterior border of our 9th segment and not near the respiratory lobes as in Roubaud's drawing.

References.

- JACKSON, C. H. N. (1948a). Proc. R. ent. Soc. Lond., (A) **23**, pp. 36-38.
 JACKSON, C. H. N. (1948b). Bull. ent. Res., **39**, pp. 441-451.
 NEWSTEAD, R., EVANS, A. M. & POTTS, W. H. (1924). Guide to the study of tsetse-flies. Liverpool. 268 pp.
 ROUBAUD, E. (1909). La *Glossina palpalis*: sa biologie, son rôle dans l'étiologie des trypanosomiasés. (In Martin, G., Leboeuf & Roubaud, E. Rapport de la mission d'études de la maladie du sommeil au Congo Français (1906-1908).)

A LIFE-HISTORY STUDY OF THE BROWN HOUSE MOTH,
HOFMANNOPHILA PSEUDOSPRETELLA (STAIN.) (LEP.,
 OECOPHORIDAE).

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(PLATES XII–XIV.)

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The Oecophorid moth, *Hofmannophila pseudospretella* (Staint.) (*Borkhausenia pseudospretella* (Staint.) of some authors) is very widely distributed in Britain. It probably occurs in small numbers in almost every private house in the country, and few warehouses, stores or mills are without a small population of this species. O'Farrell & Butler (1948) state that during the period 1942–1946 *H. pseudospretella* and *Endrosis lactella* (Schiff.) were by far the commonest warehouse moths in Northern Ireland. The same authors are of the opinion that the rôle of *H. pseudospretella* is usually that of an omnivorous scavenger in cereal spillage, dust etc., but that it occasionally becomes a major pest and attacks bulk wheat, bagged flour and a variety of stored commodities. Its importance in the home is as a clothes moth rather than as a pest of stored foods. It occurs in small numbers in the open air, and birds' nests, either in houses or in the open, may constitute an important reservoir of this species. Although Hughes (1948) states that it may be a more serious pest than the Common Clothes Moth, *Tineola bisselliella* (Humm.), and quotes Richardson (1897) who describes it as "the greatest general pest", it has, rather, a considerable nuisance value and seldom assumes the importance of a major pest. However, the occasional serious outbreaks that occur are a warning that, if conditions are suitable, it is capable of giving rise to a major infestation. The conditions likely to favour the occurrence of a serious infestation will emerge from the data presented in this paper.

The literature on this species is confined entirely to taxonomy, general accounts of the life-history, and records of damage to various materials. No investigation of the life-history, general biology and behaviour of this species under controlled conditions has been reported. The most recent general account is that of Hughes (1948) and an earlier publication of similar scope is that of Laing (1932), while some useful information is given by O'Farrell & Butler (1948). Details of the taxonomy of the adults are to be found in Corbet & Tams (1943), of the pupae in Hinton & Greenslade (1943) and of the larvae in Hinton (1943a). Records of damage to various commodities are extensive, and include Adkin (1932), Chrystal (1932) and Haines (1932) on bookbindings, Waters (1929) in birds' nests, Lepesme (1938) in dried mint, Cameron (1938) on corks of wine bottles, Hinton & Greenslade (1943) in bird guano in a metal bin, Corbet & Tams (1943) on stored cereals and dried fruit, Hinton (1943b) on dead insects in or near spiders' webs, Richards & Waloff (1947) in bulk wheat, Parkin (unpublished) on furniture and furnishings, and Musgrave (1947) on hides, leather and furs.

The Egg.

The eggs of *Hofmannophila* (see Pl. XII, fig. 1) are oval in shape, tapering slightly towards one end. They are hard and shiny, and if an attempt is made to crush one with a dissecting needle, the egg will usually spring, undamaged, from beneath the needle point. The surface is marked by shallow, parallel, longitudinal grooves, with fine transverse lines connecting them. They are laid singly, and are not found adhering to each other.

In size and colour the eggs are very variable. Some females lay batches in which the eggs are reasonably uniform, while in other cases it is possible to separate the eggs from a single female into two extreme types with a number of intermediates. A batch is sometimes found to consist largely of one of the extreme types, with only a few of the other. These two extremes may be described as small and white as opposed to large and yellow, and the latter are also somewhat more opaque than the former. These differences are not transitory, but persist throughout the incubation period. The extreme types in each mixed batch do not always differ to the same extent, but measurements were made from two typical batches. Each was divided into the two types, and ten eggs of each type from each batch were measured with a micrometer. Eggs of each type, including the intermediate type, were then weighed in groups of 50, eight different groups of each type being used, the eggs being derived from five different females. These figures are shown in Table I.

TABLE I.
Dimensions and Weight of the three Egg Types.

Egg Type		Length in mm. Mean of 20	Breadth in mm. Mean of 20	Weight of 50 Eggs in mg. Mean in 8 Groups	Weight per Egg mg.
Small White	...	·490	·340	1·01	·020
Intermediate	...	—	—	1·59	·032
Large Yellow	...	·595	·395	3·24	·065

Eggs containing two embryos are rare, but several are sometimes laid by one female in a batch of otherwise normal eggs. Very rarely a female will lay eggs of which a large proportion are double eggs. These are seldom completely viable, but sometimes one embryo develops and the other end of the egg collapses. Six double eggs were obtained in which both embryos developed; of these, five were eggs of

normal breadth but nearly twice the normal length, and a larval head capsule developed at each end. In the sixth egg, the three dimensions were all greater than normal, and two head capsules developed at the same end. In all these eggs except the last embryonic development appeared to be normal and complete, but no living larvae emerged. In one case the two larvae died as a result of mutual damage caused by their efforts to break out of the egg-shell, and it seems likely that this would usually occur in such eggs.

The Incubation Period.

The length of the incubation period was measured over a wide range of conditions. The temperature was controlled by the use of constant temperature rooms or ordinary electric biological incubators. Relative humidity was controlled by the use of potash solutions in desiccators or in small, wide-mouthed, stoppered jars for small experiments. The eggs were contained in 2 ins. $\frac{1}{2}$ in. specimen tubes with perforated corks covered with a layer of muslin. Potash solutions were made up, using hydrometers, to Solomon's modification (unpublished) of Buxton & Mellanby's figures (1934). The specific gravity at 15°C. of the various potash solutions is given in Table II.

TABLE II.

Relative Humidity and Specific Gravity at 15°C. of Solutions of Potassium Hydroxide.

R.H. %	Sp. Gr.	R.H. %	Sp. Gr.	R.H. %	Sp. Gr.	R.H. %	Sp. Gr.
95	1.062	75	1.211	55	1.310	30	1.424
90	1.105	70	1.238	50	1.330	25	1.449
85	1.147	65	1.263	45	1.352	20	1.480
80	1.182	60	1.289	40	1.377	15	1.510

Additional low relative humidities were obtained by using sulphuric acid (May & Baker) of specific gravities 1.6, 1.7 and 1.84 giving a relative humidity of 8.5 per cent., 3.2 per cent. and approximately 1 per cent. respectively. A relative humidity of approximately 0 per cent. was obtained with phosphorus pentoxide.

Eggs were collected from three females over a period of twelve hours, and 20 eggs from each female were incubated at each set of conditions. Eggs were examined at 9 a.m. and 6.30 p.m. daily, and the incubation period was measured to the nearest half-day. The slight differences between the eggs of the three different females were eliminated by this half-day approximation, except at low temperatures at which the incubation period is long and variations between the eggs of one female

TABLE III.

The Mean Length of the Incubation Period in Days for Sets of 60 Eggs under various Conditions of Temperature and Humidity.

R.H. %	Temperature in °C.							
	10	13	15	20	25	27	29	30
	Days	Days	Days	Days	Days	Days	Days	
90	110.4	42.3	25.1	14.25	9.8	8.5	10.1	Failed to hatch
70	—	—	26.0	15.4	10.1	—	—	—
50	—	—	27.6	16.05	10.4	—	—	Failed to hatch
30	—	—	29.0	18.8	12.0	—	—	Failed to hatch
15	—	—	34.6	19.2	12.5	—	—	Failed to hatch
8.5	—	—	—	—	14.0	—	—	—

and another, and between individual eggs from the same adult, are more obvious. Whereas at 25°C. the whole group of 60 eggs at any one humidity would hatch over a period of about 18 hours, at 15°C. this period might be as long as 48 hours. The results of this experiment are given in Table III. It will be seen that the eggs are highly sensitive to temperature but comparatively insensitive to relative humidity. The effect of temperature upon the length of the incubation period at a constant relative humidity of 90 per cent. is shown in fig. 2.

A further experiment was performed to determine whether the length of the incubation period was dependent upon the conditions under which the adults were kept prior to, and during, oviposition. Eggs were collected from single females laying under various controlled conditions. The eggs used were all laid during a period of 12 hours, and they were incubated in batches of ten as shown in Table IV. The incubation periods were measured to the nearest half-day. It will be seen that the length of the incubation period is not significantly affected by the conditions under which the adults are kept prior to oviposition.

TABLE IV.

The Incubation Period of Eggs under various Conditions of Development and Incubation.

Group 10 Eggs	Conditions of Development		Conditions of Incubation		Incubation Period. Days
	Temp. °C.	R.H. %	Temp. °C.	R.H. %	Mean of 10
1	10	90	25	90	9.75
2	25	90	25	90	9.9
3	25	15	25	90	9.6
4	25	90	25	90	9.7
5	25	90	15	90	24.8
6	15	90	15	90	25.6
7	25	90	25	15	12.1
8	25	15	25	15	12.45

Percentage Survival.

During the experiments on the length of the incubation period, observations were made on the proportion of larvae emerging successfully under the various conditions. This was fairly constant over the greater part of the temperature and humidity range, but at the extremes of the range the proportion of successful emergences diminished rapidly. Over the whole range of relative humidity shown in Table III, and over the temperature range 13–27°C., the minimum hatch was 16 out of 20, and 100 per cent. hatch was of frequent occurrence. For the 1,080 eggs used in the incubation period experiments a total survival of 92 per cent. was obtained over a temperature range of 13–27°C. and a humidity range of 8.5–90 per cent. R.H. Those eggs which failed to hatch almost invariably showed no signs of embryonic development.

Additional experiments were performed to investigate the percentage survival under extreme conditions of temperature and humidity. When testing the effects of extreme humidities, a moderate temperature (20°C.) was used throughout and, for the extreme temperatures, the optimum relative humidity of 90 per cent. was used in each case. The incubation periods of 60 eggs were measured under each set of conditions. The eggs were incubated in 2 ins. \times $\frac{1}{2}$ in. tubes in groups of 20, and at each examination any newly hatched larvae were removed to prevent them from eating the remaining unhatched eggs. The chances of this occurring were minimised by incubating no more than 20 eggs in any one tube. The results of these experiments are given in Tables V and VI.

TABLE V.

Percentage Survival of Eggs at Extremes of Temperature Range at Constant Relative Humidity (90 per cent.).

Temperature (°C.) ...	10	25	26	27	28	29	30	31	32
Incubation Period (Days) ...	110	9.5	9.3	8.5	8.7	10.2	—	—	—
Survival (per cent.) ...	13.3	96.7	100	76.7	65	23.3	0	0	0

TABLE VI.

Percentage Survival of Eggs at Extremes of Relative Humidity Range at Constant Temperature (20°C.).

Relative Humidity (per cent.)	0	1	3.2	100
Incubation Period (Days) ...	22.4	20.7	22.0	15.6
Survival (per cent.) ...	6	10	72	.5

Those conditions under which survival was very low —30°C., 90 per cent. R.H.; 0 per cent., 1 per cent., and 100 per cent. R.H. 20°C.—were tested many times because different batches of eggs behaved differently. Sometimes no larvae emerged successfully, and the survival given in the tables for these conditions represent the maximum recorded for any one batch. The low survival at 100 per cent. R.H. 20°C. was due to the rapidity of fungal and bacterial attack under these conditions, and at 10°C.—90 per cent. R.H. the long incubation period (110 days) resulted in the destruction of a high proportion of the eggs by moulds. In the former case a high proportion of the eggs showed signs of embryonic development before they were destroyed, but in the latter most of those destroyed showed no signs of development. It is probable that the developmental threshold for the egg lies not far below 10°C. Although no larvae emerged above 29°C. complete and apparently normal embryonic development occurred up to 32°C.

It must be emphasised that the percentage survival measured in the previous experiments is applicable only to a small fraction of the egg output of a single female, and it is probably of importance that the eggs were always collected during the early part of the oviposition period. During the course of some experiments on oviposition (to be described later) it was necessary to collect the total egg output of a large number of individual females. Some of these batches were set aside and the percentage survival determined for the total egg production of individual adults. Observations were made on 18 batches, and the survival varied from 56 per cent. to 97 per cent. with an average value of 83 per cent. In every case the eggs were laid and incubated at 25°C. and 70 per cent. R.H. Those eggs which did not hatch had usually failed to develop and very few fully developed embryos failed to emerge successfully. The factors affecting fertility, and the causes of low viability, have not been investigated.

Throughout these experiments, uniform batches of eggs were used, because of the possibility that there might be a physiological difference between the two types in a mixed batch. In order to investigate this point a number of mixed batches, laid by single females over a period of twelve hours, was obtained. Each was divided into the three types and the incubation period of each type was determined. The results were inconclusive, but on several occasions the large yellow group hatched, on the average, 48 hours before the small white group derived from the same adult. There was a slight tendency for the large yellow group to hatch early in most cases, but the difference was usually less than 12 hours. It is not known whether any differences occur during the rest of the developmental period when the larvae from the two types are reared separately.

The Larva.

The Feeding Larval Stage.

(a) Development on middlings under controlled conditions.

This series of experiments was performed to determine the length of the larval feeding period and the effect upon it of variations in temperature and relative humidity, the limits within which larval development could be successfully completed and the number of instars.

The foodstuff, middlings, was partially sterilised by heating to 60°C. for a period of 6 hours, and, before use, was exposed in a flat dish over a humidity solution of appropriate strength for 24 hours. Conditions were controlled as described in the last section. The newly hatched larvae (see Pl. XII, fig. 1) were confined in 2 ins. $\frac{1}{2}$ in. specimen tubes, closed by perforated corks and muslin. When approximately half-grown they were transferred to 3 ins. \times 1 in. specimen tubes. The corks of these tubes were protected by wire gauze because the wandering larvae will penetrate a layer of muslin and half an inch of cork in a few hours. Recently special wide-hole rubber bungs with a built-in wire gauze barrier across the hole have been available. Food was always in excess but not large excess, as mould growth is extensive at high humidities if a considerable surplus of food is present. Towards the end of the feeding period, rolls of corrugated paper were provided in which the larvae could spin their cocoons.

The feeding larva does not normally emerge from the food mass (unless short of food or overcrowded) until it is fully grown. Consequently, the sudden appearance of silk on the sides of a tube is a fairly reliable indication that at least one of the contained larvae has reached the wandering stage. Unfortunately, the exact times of wandering of the more slowly growing larvae in each tube are not so easily determined. The feeding larva spins a closely-woven tube of silk as it eats its way through the food mass, and it moults in slight enlargements of this tube. It is, on the whole, economical in the use of silk, and only spins a large quantity if confined in an empty tube. The duration of the wandering period has not been precisely investigated, but it seems to depend principally upon the availability of a suitable site for a cocoon. It normally lasts from 12 to 48 hours but, in the absence of corrugated paper or other suitable site, wandering may last a week before the larva spins up in the food. At this stage the larva measures, on the average, 18-20 mm. and weighs 75-100 mg. The largest larva recorded measured 24 mm. and weighed 130 mg.

When roughly handled the larva secretes from the head region, a fluid which varies in colour from pale amber to dark brown, and may be produced in considerable quantity if a number of larvae are confined together in an empty tube.

Table VII summarises the results of two experiments to determine the larval feeding period under various physical conditions. In each experiment a group of

TABLE VII.

Mean Duration in Days of Larval Feeding Period under various Physical Conditions.

R.H. Per cent.	Temperature in °C.				
	10	13	15	20	25
90	>180 days	145 days	126 days	78 days	71 days
80	—	—	167 days	110 days	92 days
70		No Larvae			
60		Completed Feeding Period			

12 larvae—2 tubes of 6—was used for each set of conditions. There is a certain amount of variation in the rate of growth of different larvae in the same tube due to individual peculiarities and, probably, to varying degrees of mutual interference. The last larva to wander in any one group did so up to 10 days after the first at the higher temperature, and up to 3 weeks later than the first at the lower temperatures. The duration of the feeding period for one group was taken as the mean of the individual periods.

The effects of temperature at constant relative humidity (90 per cent. R.H.) are illustrated in fig. 2.

The results show very clearly that high relative humidities are required for satisfactory larval growth. At the lower temperatures, larvae at 70 per cent. and 60 per cent. R.H. persisted for a very long time—up to 46 weeks at 70 per cent. R.H. and 15°C.—but were unable to grow appreciably. The newly hatched larva measures 1.5 mm. and the larva which persisted for 46 weeks at 70 per cent. R.H. and 15°C. measured only 6 mm. a week before it died.

Unfortunately, temperatures between 25°C. and 30°C. were available for too short a time for the complete growing period to be measured under these conditions. However, larvae were grown for as long as possible at a constant humidity of 90 per cent. R.H. and were weighed when 38 days old. The average weight of the eight larvae at each temperature, except at 29°C. at which there were only 2 larvae surviving, is given below. These figures indicate that temperatures above 25°C. have an adverse effect upon the rate of growth of newly hatched larvae at 90 per cent. R.H.

Temperature °C. ...	25	26	27	28	29	30
Mean Weight mg.	21.6	19.8	14.3	6.0	1.3	No Survivors

The number and duration of the larval instars was determined by rearing isolated larvae in 2 ins. $\frac{1}{2}$ in. tubes on the minimum amount of excess food. Since the larvae live and moult inside their silk tubes observation of moults and recovery of head capsules was impossible for any one larva, and very laborious if many were to

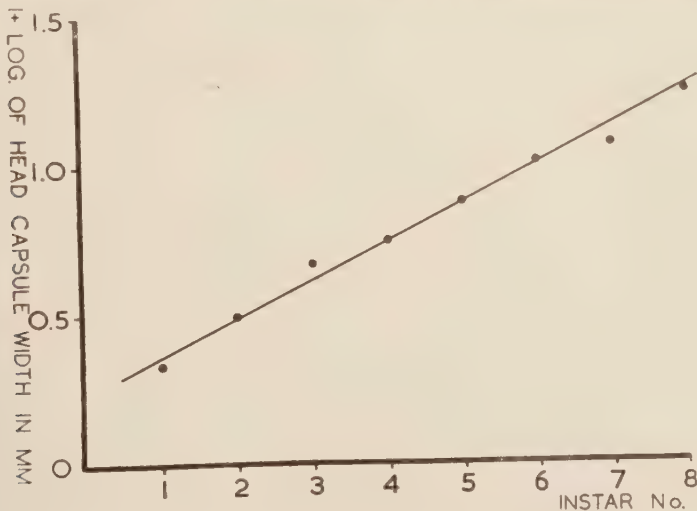


Fig. 1.—Diagram illustrating approximation to Dyar's Law.

be used. Finally, four were kept under observation at 90 per cent. R.H. and 25°C. and it was found that at least one of the four moulted against the glass or threw out its head capsule in a conspicuous place at each moult, and that, on those occasions when two did so, the two moults occurred within 24 hours of each other. Thus it was possible to determine the duration of each instar with reasonable accuracy, although the complete set of data was compiled from observations on four different larvae. The head capsules recovered were measured with a micrometer, but, because of differences in size between different larvae at the same moult, no satisfactory figures could be obtained. The experiment was therefore repeated with isolated larvae fed upon pieces of woollen cloth dusted with brewers' yeast. Growth was somewhat slower under the same conditions (90 per cent. R.H. 25°C.) but the number of instars was the same, and the head capsules were comparatively easy to collect. In one case a complete series of capsules was obtained and the widths were measured with a micrometer. A graph of the logarithm of the head capsule width plotted against the instar number approximates to the linear form required by Dyar's Law (see fig. 1). These results are given in Table VIII. No measurement could be made on the final head capsule, because this is split in two at pupation and thrown backwards attached to the rest of the cuticle.

TABLE VIII.
Number and Duration of Larval Instars on a Diet of Middlings at 90 per cent. R.H. and 25°C. Head Capsule Widths of a single Larva on a Diet of Wool and Yeast at 90 per cent. R.H. and 25°C.

Instar Number	1	2	3	4	5	6	7	8	9
Duration in Days	7	7	5.5	9.5	10.5	11.0	10.0	Highly variable	
Capsule Width mm.2150	.3125	.4690	.5940	.750	1.025	1.1875	1.740	—

The larva which wanders, spins a cocoon, and enters diapause after having been reared at 90 per cent. R.H. and 25°C. is in its 8th instar. The 8th moult occurs during diapause, and it is not possible here to give any figure for the duration of the

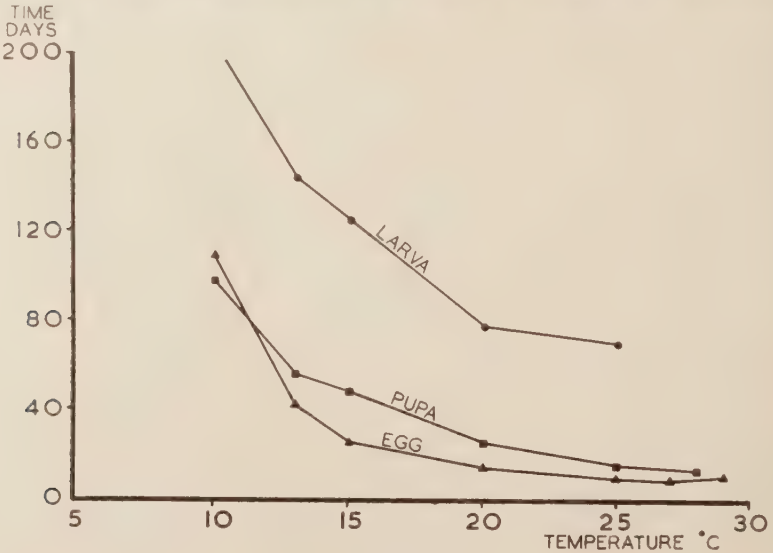


Fig. 2.—The effect of temperature on the duration of the egg, larval and pupal stages at 90 per cent. R.H.

8th and 9th instars. This point will be considered in detail in the section on diapause. It was noted that many more than 8 moults occurred during the feeding stage when larval growth was checked by a fall in relative humidity. The small larvae which persisted for long periods at 70 per cent. and 60 per cent. R.H. without appreciable increase in size moulted repeatedly during that time.

(b) *Development on various foods under controlled conditions.*

This series of experiments was designed to show what variation in the length of the larval feeding period occurred when the larvae were reared upon a variety of foodstuffs. *Hofmannophila* attacks a great variety of different materials, so a very wide range of foods was selected for testing. As already explained, the wandering of larvae other than the first in each tube is difficult to observe, so, for the purpose of comparing the different diets, the date of wandering of the first larva in each tube was taken as the end of the growing period. Each foodstuff was tested with 15 larvae, in two tubes—7 and 8 in each—so two first wanderings could be recorded for each food. The mean of these two periods was used as the duration of the larval feeding period for the purpose of comparing the different diets. Tubes contained about 4g. of foodstuff, with about $\frac{1}{2}$ per cent. by weight of sodium o-phenylphenate (B.D.H. Reagent) to control mould growth. Preliminary tests with this substance had indicated that $\frac{1}{2}$ per cent. by weight had no apparent effect upon the insects. Unfortunately, control of fungi by this method was only partial when foods were incubated at 90 per cent. R.H. for long periods. It is necessary, therefore, when considering the results of these experiments, to bear in mind that a certain amount of mould growth was probably always present, and that this may have been of importance in the case of a food which was deficient in vitamins, allowing a more rapid development than would otherwise have occurred. The range of foodstuffs tested is shown in Table IX. In all cases where yeast was added, approximately

TABLE IX.

Duration of Feeding Period and Survival of Larvae reared on various Foods at 90 per cent. R.H. and 20°C.

Foodstuff	Survival Per cent.	Feeding Period Days	Remarks
Wheatgerm	100	64	
Whole Wheat	100	68	
Beans	100	78	Some damaged beans present.
Feathers+Yeast	100	78	
Fishmeal+Yeast	100	86	
Macaroni	100	110	
Fishmeal	100	126	
Flannel+Yeast+Cholesterol	100	180	Impregnated yeast : cholesterol=10 : 1.
Peas	93	75	
White Flour	93	77	
Middlings	93	80	
Wool+Yeast	80	90	Fine woollen cloth, dusted with yeast.
Dead Moths	73	54	Adults of <i>Hofmannophila</i> and <i>Endrosis</i> .
Oats	60	95	Some damaged oats present.
Cork+Yeast	60	124	Powdered technical cork. Larvae small.
Dried Grass	60	100	Prepared commercially.
Yeast	53	130	Glaxo debittered.
Soya Flour	47	84	
Dried Milk	40	124	Glaxo "Ostermilk" No. 1.
Dried Egg	33	68	M.o.F.
Groundnuts	33	130	Shelled and broken.
Leather+Yeast	33	224	Fine shreds and small pieces.
Cornflour+Yeast	20	108	Larvae very small.

5 per cent. by weight of dried brewers' yeast (Glaxo, debittered) was mixed with the foodstuff. The purpose of this was to supply sufficient vitamins without otherwise increasing the food value of the diet.

The proportion of larvae reaching the wandering stage is important when assessing the suitability of various diets, and survival figures are also given in Table IX.

In addition to the foods listed in the table, cocoa and silk and yeast were also tested. In both cases very small larvae persisted after 250 days but it was unlikely that any would become fully grown. The silk used was fully prepared parachute silk in shreds. It appeared to pass through the larvae unchanged, and such growth as was achieved was probably due to the 5 per cent. of yeast present. It is not understood why the larvae were unable to develop satisfactorily upon cocoa.

In the case of beans and oats it was necessary to have some damaged material present as the young larvae could make no impression on the intact seeds. This was also true, to some extent, of wheat and peas, but these were more susceptible to attack than beans or oats. Usually one or two of the larvae found positions of mechanical advantage, and once an impression had been made the other larvae could make progress.

It will be seen that the most rapid development occurred upon dead adults, while the slowest was upon leather. Combining speed and survival, wheatgerm appears to be the most satisfactory diet.

It was considered possible that a sudden and complete change in the nature of the diet of growing larvae might have an adverse effect upon their rate of development. To test this possibility two groups of larvae were reared at 90 per cent. R.H. and 25°C. one upon whole wheat, and the other upon wool and yeast. After 40 days one-half of the larvae growing upon wheat were transferred to wool and yeast, and one-half of those upon wool and yeast were transferred to wheat. The larvae which had been transferred suffered no visible check to their development, and the transferred groups became fully grown later than the group which remained upon wheat, but earlier than that which remained on wool and yeast. This difference could be attributed to the superiority of wheat over wool and yeast as a diet and there was no evidence to support the supposition that the change from a vegetable diet containing much carbohydrate to one consisting largely of animal protein of poor nutritive value had, in itself, any adverse effect upon the rate or development of the larvae.

An experiment to demonstrate the growth of *Hofmannophila* larvae on a "synthetic diet" of the type described by Fraenkel & Blewett (1943) was invalidated by failure to prevent mould growth.

The Diapausing Larval Stage.

The facts of the diapause, so far as they have been elucidated, will become apparent as the various experiments are described, but a brief general description at this stage may be helpful.

Normally, the mature larva remains within its cocoon until it emerges as an adult. After spinning up it remains normal in appearance until the diapause moult, which occurs after a period the length of which depends upon the conditions. Until this moult, the larva, removed from its cocoon, is indistinguishable from a wandering larva. If, however, it is removed later in the diapause, after the diapause moult has occurred, it has a different appearance (see Pl. XIII, fig. 2). The chitinised parts, particularly the head capsule, are pale amber in colour instead of the usual dark chestnut brown. The fat body which, previously, had been visible only as a vague, translucent mass, has condensed to form a white, opaque, sponge-like structure, the lobules of which form a characteristic pattern in each segment. This mass is

divided in the mid-dorsal line by the dorsal blood vessel, which is often outlined by a dark-brown deposit. The larva is fully mobile, but its movements are slow, and it quickly spins another cocoon, usually noticeably less tough than the first. Sometimes, after the diapause moult, the larva moves forwards, spinning silk as it goes, and forms a new cocoon in front of, or partly inside, the old. Very rarely, wandering may occur at this moult, the larva spinning up again in a new situation.

About a week before pupation occurs the diapausing larva passes gradually into an ill-defined prepupal stage (see Pl. XIII, fig. 2). The thoracic limbs are extended anteriorly, the head drawn downwards, and the larva becomes progressively less mobile. Finally, the first and last two or three segments become completely transparent, the ocellar pigment is withdrawn, and the only movement is the sudden lateral bending that is characteristic of the pupa.

The literature contains no reference to a diapause in *H. pseudospretella*, and the foregoing account leaves most of the questions unanswered. When the facts are considered in relation to those described for other species, the problem inevitably arises as to precisely what constitutes diapause, and whether the behaviour of this species can be described as a true diapause.

Wigglesworth (1939) distinguishes between "quiescence" and "diapause". Quiescence is a state of arrested development due directly to the influence of unfavourable external conditions, and is brought to an end by a return to a favourable environment. He quotes Henneguy as proposing the term "diapause" for a spontaneous arrest of development not directly attributable to the effect of environmental conditions. This conception of diapause accepts as its criterion the spontaneity or otherwise of the onset of dormancy and, by implication, infers an "inborn rhythm" of development. It is doubtful if an example of this "true diapause" exists, because, as Wigglesworth points out, the environmental factor may be overlooked if its action is separated in time from the response, and thus a false impression of an internal rhythm is created. The distinction between diapause and quiescence on this basis seems to be merely a division into two types of response to external conditions, the first an immediate, and the second a delayed, response. Since these two types represent only the extremes of a graded series (Wigglesworth, 1939) it is questionable whether any sub-division is desirable, but if it is, then the most appropriate criterion to apply would seem to be the temporary irreversibility of the diapause as opposed to the complete reversibility of quiescence. On this basis, *H. pseudospretella* may be said to undergo diapause.

Some authors favour a very much wider use of the term. Squire (1940) working on diapause in the pink bollworm of cotton, *Platyedra gossypiella* (Saund.) found that diapause was induced by food or low water-content, and could be terminated by moistening the diapausing larvae. It is difficult to classify this type of behaviour as either quiescence or diapause. Squire himself is of the opinion that "hibernation, aestivation and diapause are, in reality, different manifestations of the same phenomenon which we may call diapause" and he states also that "all the remote causes of diapause boil down, in physiological terms, to an unfavourable free-water balance." In the present state of our knowledge this latter statement cannot be accepted without reservations, but if, as seems likely, it is found that control of diapause in many species is fundamentally similar, the wide view taken by Squire will have to be accepted, and the graded series between quiescence and diapause regarded as variations of a single phenomenon.

(a) *The diapause of larvae reared under field conditions.*

The larvae used in this investigation were obtained from a bin of dried grass (see Pl. XII, fig. 2) which, for several years, had been stored in an unheated outhouse with the loose-fitting lid tied down. It had been completely undisturbed, and when it came into the writer's possession was heavily infested with *H. pseudospretella* and

Endrosis lactella, with a few *Borkhausenia fuscescens* (Haw.). The precise history of the larvae was unknown, but they must have experienced some fluctuations of temperature and humidity, although probably only over a moderate range. It is unlikely that they would have experienced winter temperatures except when nearly full-grown, and their growing period was probably between 6 and 8 months. Also, it is probable that conditions within the bin at any time were reasonably uniform. These considerations justify the assumption that was made for the purpose of this experiment—that all larvae wandering at a given time were in approximately the same physiological state. In order to minimise the errors to which this might give rise, only those larvae with no food visible in the alimentary tract were selected. The frass from larvae feeding upon dried grass is black, so this selection was not difficult.

Four hundred wandering larvae were taken from the bin over a period of 5 hours, and divided into groups of 100 each. Rolls of corrugated paper were provided in which the larvae could spin up, and the groups were incubated at 25°C., 20°C., 15°C. and 9–12°C., the relative humidity being approximately 70 per cent. in each case. Most of the larvae had disappeared into the paper rolls after a few hours, but a few continued to wander for up to 2 days, most of them at 9–12°C. The period between the start of the experiment and the emergence of the first and last adults respectively, less the duration of the pupal stage in each case, gave figures for the minimum and maximum duration of the diapause at each temperature (see fig. 3).

(b) *The diapause of larvae reared under controlled conditions.*

This was not investigated specifically by a large-scale experiment as has been described in the previous section, but data were obtained from the initial experiments to determine the life-history on middlings. As in the previous experiment the dates of pupation were deduced from the dates of emergence of the adults, the duration of the pupal stage being reasonably constant at a given temperature. The duration of the diapause under these conditions is shown diagrammatically in fig. 3.

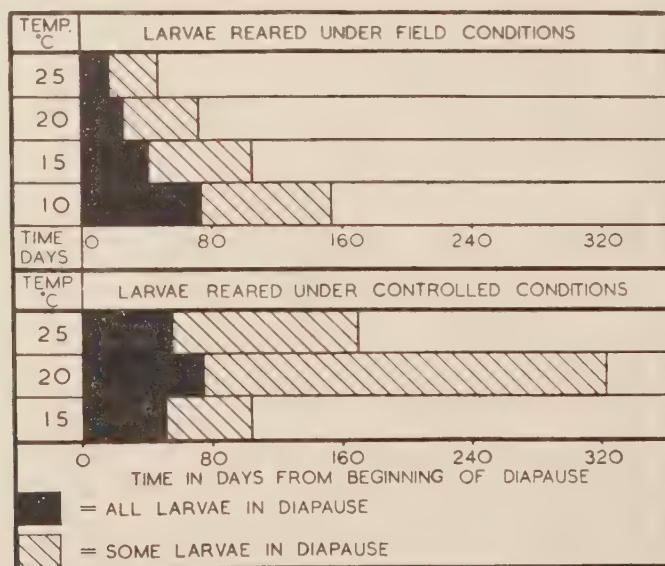


Fig. 3.—The effect of temperature during diapause upon the length of the diapause. Field Conditions=Variable temperature and humidity and diet of dried grass. Controlled conditions=Temperature as for diapause, humidity 90 per cent. R.H., and a diet of middlings.

These two experiments demonstrated that, in the case of field-reared larvae which had grown comparatively slowly, and had experienced fluctuating conditions, the length of the diapause and the degree of variation between individuals at the same temperature were much reduced in comparison with culture-reared larvae diapausing under identical conditions. The field group as a whole responded normally to differences in temperature during diapause. Life processes being slower at the lower temperatures, diapause was prolonged and the variation at the same temperature was exaggerated compared with the higher temperatures. This was also true of the culture-reared group as a whole except that at a constant temperature of 15°C. both the length of the diapause and the degree of variation were conspicuously reduced. Apparently the temperature conditions represented by a constant temperature of 15°C. throughout life were equivalent in effect to the conditions experienced by the field larvae and the diapause therefore corresponded approximately in length and degree of variation.

The conditions of growth of the cultured larvae differed from those of the field larvae in respect of one additional factor. Whereas the latter had been fed on dried grass, the former had been reared upon a diet of middlings. However, it was established during the experiments on various foodstuffs that the nature of the diet had no apparent effect upon the duration of the diapause except that those larvae which had been fed upon a diet of poor nutritive value and were consequently small when they reached the wandering stage, tended to pupate somewhat earlier than usual. This may have been a direct effect of the diet, or a natural consequence of small size; this point will have to be investigated.

A separate experiment was carried out to determine whether the duration of the diapause in field-reared larvae was influenced by the relative humidity during diapause. Five humidities between 95 per cent. R.H. and approximately 1 per cent. R.H. were used at 25°C., 20°C., and 15°C. The diapause moult occurred first at the lowest humidity and the highest temperature, its occurrence passing regularly up each humidity series. Pupation occurred at all humidities except 1 per cent. R.H.; but no connection could be established between the length of the diapause and the relative humidity. At 1 per cent. R.H. the larvae survived for 3 months, and although continuously exposed to desiccation by sulphuric acid (Sp. Gr. 1.84) moulted successfully four times during that period, and several began to pupate, but became desiccated during the process.

*These experiments on diapause give rise to two major problems.

I. What is the factor (or factors) which tends to produce and prolong or reduce and eliminate the diapause, and how and when does it act?

II. What is the cause and significance of the immense variation which occurs between individuals treated in an identical manner under controlled conditions?

The progress which has been made towards answering these two questions is described in the next section.

(c) *Further experiments on diapause.*

Initially these experiments were all directed towards breaking an existing diapause—that is, causing a group of diapausing larvae to pupate within a short time. All the usual methods of breaking diapause were tried. Diapausing larvae were subjected to extremes of temperature and humidity and to immersion in water, and were exposed to low concentrations of the vapours of various volatile solvents (ethyl alcohol, ethyl formate, diethyl ether, acetone, chloroform, xylol, benzene, carbon disulphide and glacial acetic acid) (Pepper & Hastings, 1943). They were injected with substances likely to affect their rate of metabolism (methylene blue, glucose, insulin, thyroxine) and subjected to mechanical maltreatment of various kinds. In no case was it possible to say that the diapause had been broken.

The failure of all efforts to break the diapause prompts the conclusion that the factor controlling diapause acts before the larvae become full grown, and the course of events after that can only be influenced within certain limits. The results of the experiment with larvae reared under controlled conditions described in the previous section indicated that it should be possible to reduce the length of the diapause by rearing larvae at low temperatures, and this method was therefore tested. Larvae were reared from eggs at a constant relative humidity of 90 per cent., and at a partially controlled temperature which was not subject to a daily fluctuation of more than $\pm 1^{\circ}\text{C}$., but which rose gradually from 8 to 9 $^{\circ}\text{C}$. at the start of the experiment to 9–10 $^{\circ}\text{C}$. at 2 months, 10–12 $^{\circ}\text{C}$. at 4 months and 13–16 $^{\circ}\text{C}$. at 6 months. The larvae were fed on middlings, and confined in groups of 10, first in 2 ins. \times $\frac{1}{2}$ in. and later in 3 ins. \times 1 in. tubes. At 6 months, 27 fully grown larvae were removed and incubated at 90 per cent. R.H. and 25 $^{\circ}\text{C}$. They all pupated within 18 days. There was no diapause moult and the larvae never assumed the appearance typical of diapause. The diapause had been completely eliminated and the wandering larvae passed directly into the prepupal stage.

A small number of larvae was reared on middlings at 90 per cent. R.H. and 13 $^{\circ}\text{C}$. The diapause was still in evidence at this temperature, so it seems likely that a temperature below 12 $^{\circ}\text{C}$. and possibly below 10 $^{\circ}\text{C}$. is necessary at some period during larval growth if diapause is to be eliminated completely. It is possible that it is some sort of average of the temperature over the entire larval growing period which controls the length of the diapause.

No progress has been made in the investigation of the problem of individual variation in the length of diapause under constant conditions.

(d) *Discussion on diapause.*

The importance of diapause to the insect is that it provides a method of surviving adverse external conditions and, although the result is similar in the different species, the precise mechanism by which this result is achieved varies greatly from one insect to another. A number of examples is given by Wigglesworth (1939), and Waloff (1949) discusses the diapause of *Éphestia elutella* (Hb.) in comparison with a number of species. In spite of the wide diversity of detail, it is apparent that low temperature and water-balance play an important part in the diapause of most of the species in which it has been studied. In addition to the examples discussed by the two authors mentioned, Salt (1947) has examined in some detail the breaking of the diapause in the wheat-stem sawfly, *Cephus cinctus* Nort., by low temperature. Squire (1940) found that water-relations were the critical factor in the diapause of *Platyedra gossypiella* (the pink bollworm of cotton), and the water-content of the food was also critical in *Loxostege sticticalis* (L.) (Strelnikov, 1936). It has already been seen that low temperature throughout larval growth eliminates the diapause in *H. pseudospretella*.

Many suggestions have been made concerning the physiological changes involved in the induction and breaking of diapause. Wigglesworth (1939) summarises the more important ideas, and it seems that the hormonal control of diapause is the hypothesis which deserves most attention. It is certain that the onset of pupation, which is the visible termination of diapause in full-grown larvae, is controlled by a hormone and it is possible that some sort of balance exists between the pupation hormone and the "juvenile" hormone of Wigglesworth (1947), that this balance controls the length of the diapause and that it is affected by external conditions only during a certain limited period of the insects' development. Waloff (1949) postulates the production of a diapause-inducing factor to explain the observed facts of the diapause in *E. elutella*, and suggests that it is produced mainly during the early weeks of dormancy. The amount produced depends upon the temperature at that time, and this, together with the subsequent temperature, determines the speed with which it is eliminated, and consequently the length of the diapause.

Much more work, however, will have to be done before it can be conclusively shown that the hormonal control of diapause is an established fact, and that it occurs in many different species. So far as they go, the facts of the diapause in *H. pseudospretella* are consistent with the existence of a control of this type.

The Pupa.

When first formed the pupa (see Pl. XIII, fig. 3) is nearly white, but it darkens rapidly to a deep amber colour. The eyes become pigmented after about a week and the wing-sheaths become black immediately prior to emergence. There is no difference, other than size, which is not always reliable, between the sexes in the pupal stage.

Because of the uncertain length of the diapause and the opacity of the cocoons it was always difficult to note exactly the date of formation of a pupa. The method finally adopted was to allow a large number of larvae to spin up in a roll of corrugated paper. After six weeks at 25°C. the roll of paper was torn open. The older pupae were not used, but some were still white and soft, and these were used in experiments in which it was necessary to know fairly accurately the date of pupation. Immobile prepupae also were collected, and placed in empty tubes so that their pupation could be observed.

In general, it can be said that the pupa is sensitive to temperature but not to humidity. No difference was observed between the length of the pupal stage in the two sexes. In the experiment, 20 pupae were incubated at each of three humidities at 25°C., and 20 at 90 per cent. R.H. at each of the other temperatures. The results are given in Table X and illustrated in fig. 2.

TABLE X.
Mean Duration of Pupal Stage under various Physical Conditions.

R.H. per cent.	Temperature in °C.						
	10	13	15	20	25	28	30
90	98	56	48	25	15.5	13	Failed to emerge
50	—	—	—	—	15.1	—	—
15	—	—	—	—	16.3	—	—

The Adult.

Mating.

Mating of adults usually occurred between 12 and 24 hours after emergence, and oviposition commenced within 24 hours of mating. A period of 12 hours usually elapsed between mating and oviposition, but eggs were laid almost at once if the female was mated when several days old. It was frequently noted that old males mated more readily than those which had only recently emerged. The female did not appear to adopt any characteristic attitude prior to mating, and males mated readily with etherised females. One male can successfully impregnate several females.

In the intensely crowded conditions prevailing in the mass culture in the bin of dried grass already referred to, at least 14 cases were observed of copulation between males of *H. pseudospretella* and females of *E. lactella* (see Pl. XIV, fig. 2). Several such pairs separated upon capture, several others remained in copula until they died, apparently unable to separate, and the remainder appeared to be effective matings in which the pairs broke up in the usual manner. Subsequent dissection of the

females revealed that in two cases the bursa copulatrix contained a spermatophore. The bursa of a normally fertilised female of each species was dissected out for comparison, but, with the limited material available, it was not possible to determine from which species the unknown spermatophores had derived. While it remains possible that these two *Endrosis* females which had mated with *Hofmannophila* males had previously been fertilised by males of their own species, this possibility is made less likely by the consideration that the *Endrosis* sex-ratio during the period over which the cross-matings occurred was $<1\frac{1}{2} : 20\%$. No eggs derived from any of the mixed pairs, however, showed signs of development and this also detracts from the possibility that the cross-matings had, in two cases, been preceded by normal impregnation.

Oviposition.

Adults for oviposition experiments were obtained from isolated pupae, incubated at 20°C . The moths were sexed upon emergence, the females weighed, and pairs were set up in 3 ins. \times 1 in. tubes containing a layer of whole wheat about two grains deep. The eggs were sieved off at convenient intervals and counted. Precautions were taken to ensure that only normal females were used. If no, or only a few, eggs had been laid by the fourth day the male was changed and if no eggs appeared within another 48 hours the pair was discarded. Pairs were also destroyed if the female showed obvious abnormality in laying—if, for instance, the eggs were misshapen or double, or if only one or two eggs were laid daily.

The rate at which the eggs were laid depended upon the temperature. At 20°C . and 25°C . the bulk of the egg output was laid during the first two or three days, and, on one occasion, a female laid 190 eggs between 12 and 24 hours after mating. After the first few days the daily output diminished rapidly, and only a few eggs were laid during the last week of life. At the lower temperatures, fewer eggs were laid daily at first but a moderate daily output was maintained throughout most of the laying period.

Three experiments were performed to demonstrate the correlation between the weight of a female at emergence and the total egg output, and the effect of variations in temperature and relative humidity upon the total egg number. These results are given in Table XI and illustrated in fig. 4. The statistical methods used are from Fisher (1932). It will be seen that in each experiment there is a significant

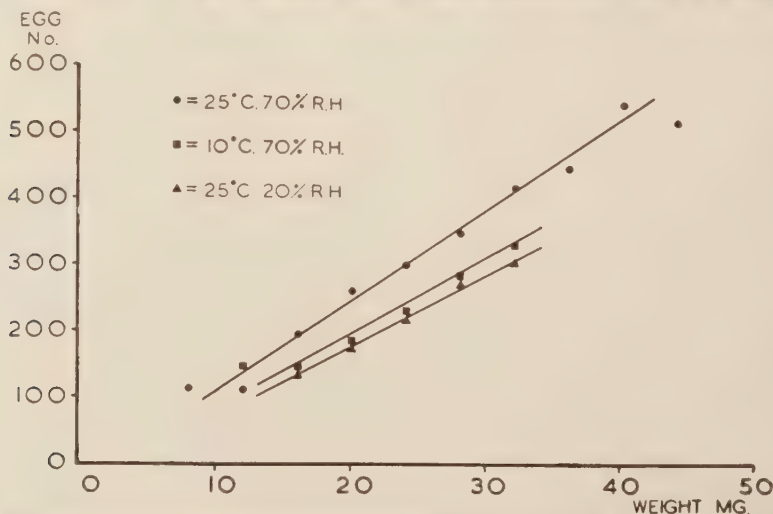


Fig. 4.—The relationship between weight of female at emergence and egg production.

TABLE XI.
Mean Egg Production of Females of different Weights under various Physical Conditions.

	Conditions	Weight of ♀ in mg.	6-10	10-14	14-18	18-22	22-26	26-30	30-34	34-38	38-42	42-46	All Weights
Experiment 1	Temp. 25°C. ...	No. of Moths ...	1	2	27	23	21	9	3	6	2	2	96
	R.H. 70 per cent.	Mean Egg No. ...	114	110	195	261	301	350	420	450	540	520	283
	Temp. 10°C. ...	No. of Moths ...	—	2	5	7	6	3	1	—	—	—	24
Experiment 2	R.H. 70 per cent.	Mean Egg No. ...	—	146	145	185	232	289	333	—	—	—	213
	t. Signif. of difference between means, Experiments 1 and 2	...	—	Not Signif.	2.76	3.05	2.95	Not Signif.	Not Signif.	—	—	—	—
	Temp. 25°C. ...	No. of Moths ...	—	—	3	5	6	3	6	—	—	—	23
Experiment 3	R.H. 20 per cent.	Mean Egg No. ...	—	—	144	187	224	286	306	—	—	—	235
	t. Signif. of difference between means, Experiments 1 and 3	...	—	—	2.08	2.21	2.68	Not Signif.	3.77	—	—	—	—
	Values of r for p=.05.												—
Correlation between Egg Production and Weight of Female at Emergence ...			Experiment 1			Experiment 2			Experiment 3				
			.6907 (Signif.)			.7566 (Signif.)			.7505 (Signif.)				

correlation between weight of the female at emergence and egg production. The difference due to temperature (comparison of Experiments 1 and 2) is significant in those weight groups containing a reasonable number of individuals, and the difference due to relative humidity (comparison of Experiments 1 and 3) is significant for all except one of the weight groups. In Experiment 1, the lightest female weighed 8.2 mg. and laid 114 eggs while the heaviest weighed 43.6 mg. and laid 563 eggs. The smallest number laid was 106 eggs by a female weighing 12.0 mg. and the largest number was 657 eggs, laid by a female weighing 40.3 mg.

A series of experiments was carried out to determine whether the female showed a marked preference for certain sites for oviposition. Isolated females were exposed over equal areas of two different materials for a period of 24 hours at 25 C. and 90 per cent. R.H., and at the end of that time the eggs laid on each surface were counted. The moths were confined above a disc of glazed cardboard by a petri dish, and two holes of 1 inch diameter cut in the card allowed exposure of equal areas of the test surfaces. A certain number of eggs was usually laid indiscriminately over the cardboard, especially round the edge of the petri dish, but only a small proportion of the total was laid in this manner. The following order of preference was established :—

Coarse sacking.

Grain (wheat or oats).

Small stones (similar to wheat in size and shape).

Fine woollen cloth.

Large seed (peas, beans, sunflower).

Stones (similar to peas in size and shape).

Wood

Brick

Muslin

} Not preferred to the cardboard background.

As an example of the numerical results obtained, Table XII gives the figures for sacking compared with whole wheat, and the comparison of other pairs was equally convincing. The fact that small stones resembling wheat were preferred to woollen cloth, peas and beans indicates that the response is primarily tactile.

TABLE XII.

Comparison of Sacking with Wheat as an Oviposition Site.

Material	Egg Number								Total
	♀ No. 1	♀ No. 2	♀ No. 3	♀ No. 4	♀ No. 5	♀ No. 6	♀ No. 7	♀ No. 8	
Sacking ...	131	89	57	188	129	122	159	230	1,105
Wheat ...	9	29	31	62	72	60	42	29	334
Loose Eggs ...	4	1	10	1	18	14	9	10	67

An additional experiment with a small dish of water beneath a comparatively large area of sacking indicated that humidity plays no part in the selection of oviposition sites.

Sex ratio.

Throughout the experimental work it was noticed that when, for instance, oviposition pairs were being set up, there tended to be a shortage of males. This tendency was confirmed when systematic counts were made, although the sex ratio was not far from 1 : 1. Adults were taken from the bin of dried grass five times at intervals of a fortnight. The total number of moths counted, 894, consisted of 487 females and 407 males. This ratio was roughly adhered to in each of the five

counts, although in the first the proportion of females was rather higher. It is possible that, to some extent, this discrepancy between the numbers of the two sexes is due to unconscious selection of the less active moths during the catching process. This certainly contributed towards the rather high proportion of females in the first count, and during subsequent counts care was taken to pursue persistently any individual selected even though its capture proved difficult, and not to abandon a particularly active male for a less active female.

Longevity.

The duration of the adult life of mated moths was determined partly by observation of the pairs set up for oviposition experiments, and partly by setting up further pairs specifically for the purpose. The information obtained is summarised in Table XIII (see also fig. 5). The correlation between longevity and weight of female at emergence is significant except in Experiment 3, and the differences due to temperature (comparison of Experiments 1 and 2) and humidity (comparison of Experiments 1 and 3) are significant for most weight groups.

Fewer observations were made on males, which were not weighed; the figures are 7.1 days (mean) at 25°C. (range four to 11 days) and 18.8 days (mean) at 15°C. (range 16 to 27 days). The relative humidity was controlled at 70 per cent. at both temperatures.

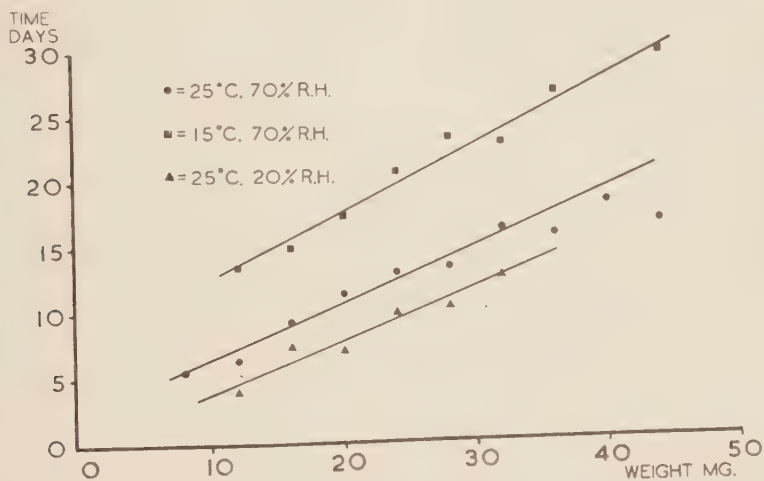


Fig. 5.—The relationship between weight of female at emergence and longevity.

The longevity of unmated adults was determined by separate experiments. In the case of the female the significance of the data is not altogether clear. An unmated female lays very few eggs early in her life, and the act of impregnation appears to be a necessary stimulus for normal oviposition. She lays more rapidly towards the end and may die after a final burst of egg production, but this latter phase does not always occur. The number of eggs laid varies greatly and apparently at random, and this almost certainly affects the length of life. If two unmated females are kept under identical conditions, one of them may lay many eggs and die quickly, while the other may lay few eggs and live a long time. Neither of these variables can be correlated with the original weights of the moths, and the cause of this variability is unknown. The point is one of minor interest only, so no analysis of the data has been attempted, but the salient points are given in Table XIV.

TABLE XIII.
Mean Longevity of Females of different Weights under various Physical Conditions.

Experiment	Conditions	Weight of ♀ in MG.										All weights
		6-10	10-14	14-18	18-22	22-26	26-30	30-34	34-38	38-42	42-46	
Experiment 1	Temp. 25°C. ...	2	3	25	23	18	8	4	6	2	1	92
	R.H. 70 per cent. ...	5.5	6.0	9.3	11.5	13.1	13.5	16.5	16.0	18.5	17.0	11.9
Experiment 2	Temp. 15°C. ...	—	2	5	13	12	5	7	2	0	2	48
	R.H. 70 per cent. ...	—	13.5	15.0	17.4	20.8	23.4	23.1	27.0	30.0	30.0	19.0
Experiment 3	t. Signif. of difference between means. Experiments 1 and 2 ...	—	Not Signif.	7.29	7.41	6.18	3.70	3.11	7.80	—	—	—
	Temp. 25°C. ...	—	2	5	8	6	8	8	—	—	—	37
	R.H. 20 per cent. ...	—	4.0	7.4	7.1	10.0	10.4	12.75	—	—	—	9.4
	t. Signif. of difference between means. Experiments 1 and 3 ...	—	Not Signif.	5.76	2.96	3.00	Not Signif.	2.60	—	—	—	—
Values of r for $\bar{p} = .05$												
		Experiment 1				Experiment 2				Experiment 3		
Correlation Between Longevity and Weight of Female at Emergence7645 (Signif.)				.6916 (Signif.)				.2295 (Not Signif.)		

TABLE XIV.

Longevity of unmated Adults under various Physical Conditions

Sex	Conditions		Weight of Adult in mg.		Egg No. per Female		Longevity in Days	
	Temp.°C.	R.H. %	Mean	Range	Mean	Range	Mean	Range
Female	25	70	23.0	14.4-38.6	152	53-300	12.0	7.0-20.0
	15	70	27.4	19.4-36.8	130	21-287	39.2	15.0-52.0
	25	20	22.4	14.5-36.3	129	54-238	7.4	3.0-10.0
Male	25	70	8.9	5.8-11.8	—	—	8.0	—
	15	70	9.8	9.3-10.6	—	—	36.0	33.0-39.0
	25	20	10.9	9.4-12.7	—	—	7.7	2.0-12.0

Parasites and Predators.

Several parasites have been reported attacking larvae of *H. pseudospretella*. They are the Ichneumonids, *Acrotomus sexcinctus* (Grav.) (Morley & Rait-Smith, 1933), *Phygadeuon bitinctus* (Gmel.) (Richards, 1949), and *Angitia chrysosticta* (Gmel.) (Richards, 1949); and the Braconid, *Ascogaster rufidens* Wesm. (Morley & Rait-Smith, 1933). None of these appeared in any of the cultures.

The most important predator was undoubtedly the mite *Cheyletus eruditus* (Schr.). It occurred in most cultures, and destroyed eggs and newly hatched larvae in considerable numbers. Many mites were seen to destroy eggs but *C. eruditus* was the only one observed to attack the young larvae. A similar observation has been reported by O'Farrell & Butler (1948). A Gamasid mite also occurred in considerable numbers in many cultures, where it fed on other mites and probably also upon eggs of *H. pseudospretella*. Up to five of these mites might be found clinging to the body of a single living moth, although one to three per moth was more usual. When adults were emerging in a culture which contained moderate numbers of these mites, 80 to 90 per cent. of the moths were found to be carrying one or more mites. No evidence was obtained that the adult moths suffered any damage, and the Gamasid was not observed to attack the young larvae as was *Cheyletus*. It has been identified as a species of *Seiulus*.

Another predator was the common spider, *Stearodca bipunctata* (L.), which was present in considerable numbers in the dried grass culture and destroyed adults and larvae of both the species present.

Discussion.

A brief outline of the general habits and distribution of *H. pseudospretella* was given in the introductory section of this paper. It is now possible to discuss more fully the field distribution and behaviour of this species.

Certain features of its life-history make *H. pseudospretella* potentially a very dangerous pest. These are its omnivorous habits, its high reproductive capacity and the resistance of all stages except the feeding larva to adverse conditions. The fact that it seldom becomes a major pest is due to the need of the larva for high humidity and the considerable length of the developmental period even under optimum conditions. Thus, even if an adequately high humidity prevails indefinitely, it is a long time before *Hofmannophila* can increase its numbers sufficiently to cause a major infestation, and stored commodities are seldom left undisturbed for the necessary time. It is normally only in spillage and debris that conditions suitable for the development of *Hofmannophila* can always be found, but as long as such local infestations exist the threat of a major outbreak will occasionally be realised.

Richards & Waloff (1947) reporting on the seasonal variation in numbers of *H. pseudopretella* breeding on bulk grain in a London granary, state that in 1943 adults were seen between late May and late September with peak emergences in early June and early August. In 1944 adults were seen between June and September with peaks in early July and late August. O'Farrell & Butler (1948) referring to the occurrence of the moth in Northern Ireland, also report two peak emergences, one in June and the other in October, but state, in addition, that all stages occur throughout the year. If it is assumed that the relative humidity remains adequately high (at or above 80 per cent. R.H.) and that normal seasonal variations in temperature occur, it is possible to speculate upon the probable cycle of generations. Adults emerging in early summer would give rise to fully grown larvae in early autumn. These, having experienced only summer temperature during their growing period, would enter diapause, pupate in late spring when temperatures began to rise and emerge as adults at the June peak. Adults emerging in late summer would give rise to young larvae which, growing slowly throughout the winter and more rapidly in spring, would become full grown in summer. Having experienced winter temperatures throughout most of their growing period they would not enter diapause, but pupate at once and emerge as adults at the late summer peak. Unfortunately, it is impossible to visualise the effect which variations in relative humidity would exert upon such a cycle. Should relative humidity fall at any time to about 60 per cent, all larval growth would be checked until it rose again, but other stages would continue their development almost unaffected. If the fall in relative humidity was prolonged, but eventually rose to a level at which larval growth was just possible, or if it fluctuated about this level, it is possible that the emergence of the adult would occur two years after the egg was laid. This problem can best be investigated by field observation and by long-term experiments carried out under field conditions. On the basis of present knowledge, it seems likely that the continuous emergence of adults over most of the year is a result of variations in humidity, and the two peaks superimposed upon this continuous emergence are the effects of seasonal temperature variations and diapause. It is most unlikely that one generation of adults could give rise to a second generation in the same year under field conditions except in isolated exceptional cases, so this alternative explanation of the regularly occurring second peak emergence need not be considered.

Summary.

Hofmannophila pseudopretella is widely distributed in this country. It is a minor pest of stored foodstuffs, clothes and furnishings, and under certain conditions may give rise to a major infestation.

The egg stage is characterised by a high sensitivity to temperature and almost complete indifference to humidity. The incubation period varied from 110 days at 10°C. (90 per cent. R.H.) to 8.5 days at 27°C. (90 per cent. R.H.) and from 9.8 days at 90 per cent. R.H. (25°C.) to 14.0 days at 8.5 per cent. R.H. (25°C.). The percentage survival was greatly reduced both at very low (<3 per cent. R.H.) and at very high (100 per cent. R.H.) humidities. The survival of eggs from a single female varied from 56 to 97 per cent. under favourable conditions.

The duration of the feeding larval stage varied between 145 days at 13°C. and 71 days at 25°C. (at 90 per cent. R.H. on middlings). Larvae failed to mature below 80 per cent. R.H. at all temperatures. Larvae were reared successfully on a wide range of diets of both animal and vegetable origin, some predominantly carbohydrate, some almost entirely protein. The most rapid development occurred on dead adults and the slowest upon leather and yeast.

Under most conditions fully grown larvae entered diapause. This was characterised by a diapause moult after which the larvae assumed a typical diapause

appearance. The length of the diapause was extremely variable and was determined largely by the temperature during larval growth. Larvae grown at low temperature did not enter diapause when incubated at 25°C. as fully grown larvae. The diapausing larvae were remarkably resistant to desiccation.

The duration of the pupal stage is affected by temperature but not by humidity. It lasted 98 days at 10°C. and 13 days at 28°C.

The total length of the developmental period was highly variable because of the variability in the length of the diapause under constant conditions. When the conditions throughout were 25°C. and 90 per cent. R.H. the total developmental period varied between 152 and 266 days, and at 20°C. and 90 per cent. R.H. the figures were 192 to 440 days. The developmental period was approximately 12 months under field conditions.

Under crowded conditions, mating was observed between males of *H. pseudospretella* and females of *Endrosis lactella* but none of the eggs developed.

The weight of the female at emergence was the most important factor governing the number of eggs laid. Weight of females varied between 8.2 mg. and 43.6 mg. and the egg number between 106 and 657 at 25°C. and 70 per cent. R.H. The number laid was significantly reduced at lower temperatures (10°C.) and at lower humidities (20 per cent. R.H.).

The sex ratio was found to be approximately 1 : 1, with a slight predominance of females.

The longevity of the fertilised adult female depended upon its weight at emergence and upon the physical conditions. Variation in weights of males was small, and the length of life of mated males could be correlated directly with the physical conditions. The mean longevity of mated females of all weights was 11.9 days at 25°C. and 70 per cent. R.H., 19.0 days at 15°C. and 70 per cent. R.H. and 9.4 days at 25°C. and 20 per cent. R.H. At 25°C. mated males lived 7.1 days on the average, and 18.8 days at 15°C.

The only important predator was the mite, *Cheyletus eruditus*, which destroyed numbers of eggs and young larvae.

Acknowledgements.

I am indebted to Mr. E. Browning and Mr. J. D. Bradley of the British Museum (Nat. Hist.) for identification of the spider, *Stearodea bipunctata*, and the moth, *Borkhausenia fuscescens*, respectively. Also to Mrs. A. M. Hughes of the Royal Free Hospital School of Medicine for identification of the mite, *Seiulus* sp.; to Mr. M. E. Solomon of this laboratory for identification of the mite, *Cheyletus eruditus*; and to Mr. J. H. Hammond, also of this laboratory, for taking the photographs.

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References.

- ADKIN, R. (1932). *Borkhausenia pseudospretella* Stt. attacking book-bindings.—Ent. mon. Mag., **68**, pp. 40-41.
- BUXTON, P. A. & MELLANBY, K. (1934). The measurement and control of humidity.—Bull. ent. Res., **25**, pp. 171-175.

- CAMERON, A. E. (1938). Insect and other pests of 1937.—Trans. Highl. agric. Soc. Scot., **1938**, repr. 35 pp.
- CHRYSTAL, R. N. (1932). An Oecophorid moth, *Borkhausenia pseudopretella* Stainton, attacking book bindings.—Ent. mon. Mag., **68**, pp. 9–10.
- CORBELL, A. S. & TAMS, W. H. T. (1943). Keys for the identification of the Lepidoptera infesting stored food products.—Proc. zool. Soc. Lond., (B) **113**, pp. 55–148.
- FISHER, R. A. (1932). Statistical methods for research workers. Edinburgh.
- FRAENKEL, G. & BLEWETT, M. (1943). The basic food requirements of several insects.—J. exp. Biol., **20**, pp. 28–34.
- HAINES, F. H. (1932). *Borkhausenia pseudopretella* Stt. attacking book-bindings.—Ent. mon. Mag., **68**, p. 68.
- HINTON, H. E. (1943a). The larvae of the Lepidoptera associated with stored products.—Bull. ent. Res., **34**, pp. 163–212.
- HINTON, H. E. (1943b). House moths feeding on dead insects in or near spider webs.—Entomologist, **76**, pp. 4–5.
- HINTON, H. E. & GREENSLADE, R. M. (1943). Observations on species of Lepidoptera infesting stored products. XI. Notes on some moths found in bird guano.—Entomologist, **76**, pp. 182–184.
- HUGHES, A. W. McK. (1948). Clothes Moths and House Moths.—Econ. Ser. Brit. Mus. (Nat. Hist.), no. 14 (4th edn.), 28 pp.
- LAING, F. (1932). *Borkhausenia pseudopretella* and other House Moths.—Ent. mon. Mag., **68**, pp. 77–80.
- LEPESME, P. (1938). *Hofmannophila pseudopretella* Stt. (Lep. Gelechiidae), hôte indésirable des habitations et des magasins.—Bull. Soc. ent. Fr., **42**, pp. 283–288. (R.A.E., (A) **26**, p. 412.)
- MORLEY, C. & RAIT-SMITH, W. (1933). The Hymenopterous parasites of the British Lepidoptera.—Trans. R. ent. Soc. Lond., **81**, pp. 133–183.
- MUSGRAVE, A. J. (1947). Entomology and the leather industry.—Progr. Leath. Sci. 1920–45, chap. 22, pp. 473–485. London, Brit. Leath. Manuf. Res. Ass.
- O'FARRELL, A. F. & BUTLER, P. M. (1948). Insects and mites associated with the storage and manufacture of foodstuffs in Northern Ireland.—Econ. Proc. R. Dublin Soc., **3**, pp. 343–407.
- PEPPER, J. H. & HASTINGS, E. (1943). Biochemical studies on the Sugar Beet Webworm (*Loxostege sticticalis* L.) with special reference to the fatty acids and their relation to diapause and sterility.—Bull. Mont. agric. Exp. Sta., no. 413, 36 pp.
- RICHARDS, O. W. (1949). Parasitic Hymenoptera found in British houses, warehouses and ships.—I: Ichneumonidae.—Proc. R. ent. Soc. Lond., (B) **18**, pp. 19–35.
- RICHARDS, O. W. & WALOFF, N. (1947). Seasonal variations in the numbers of some warehouse insects.—Proc. R. ent. Soc. Lond., (A) **22**, pp. 30–33.
- RICHARDSON, N. M. (1897). Dorset Clothes-moths and their habits.—Proc. Dorset nat. Hist. Fld Cl., **18**, pp. 138–149.

- SALT, R. W. (1947). Some effects of temperature on the production and elimination of diapause in the Wheat Stem Sawfly, *Cephus cinctus* Nort.—Canad. J. Res., (D) **25**, pp. 66–86.
- SQUIRE, F. A. (1940). On the nature and origin of the diapause in *Platyedra gossypiella* Saund.—Bull. ent. Res., **31**, pp. 1–6.
- STRELNIKOV, I. (1936). Wasserumsatz und Diapause bei *Loxostege sticticalis*. - C. R. Acad. Sci. URSS, (N.S.) 1936 **1**, pp. 267–271. (R.A.E., (A) **24**, p. 673.)
- WALOFF, N. (1949). Observations on larvae of *Ephesia chutella* Hübner (Lep. Phycitidae) during diapause.—Trans. R. ent. Soc. Lond., **100**, pp. 147–159.
- WATERS, E. G. R. (1929). A list of the Micro-Lepidoptera of the Oxford district.—Proc. Ashmol. nat. Hist. Soc., **1928**, 2nd pag., 72 pp.
- WIGGLESWORTH, V. B. (1939). The principles of insect physiology. London.
- WIGGLESWORTH, V. B. (1947). The corpus allatum and the control of metamorphosis in insects.—Nature, **159**, p. 872.
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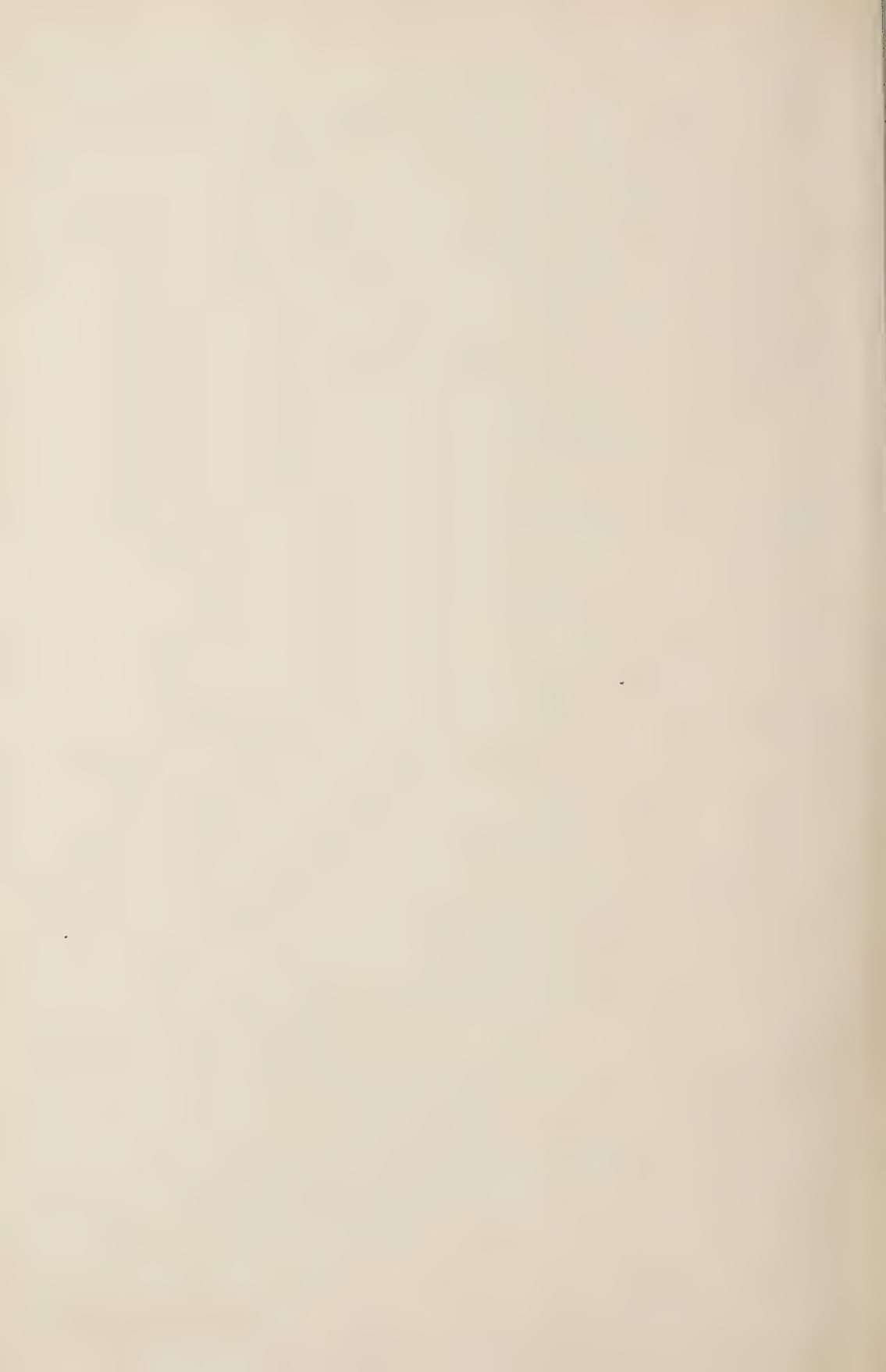
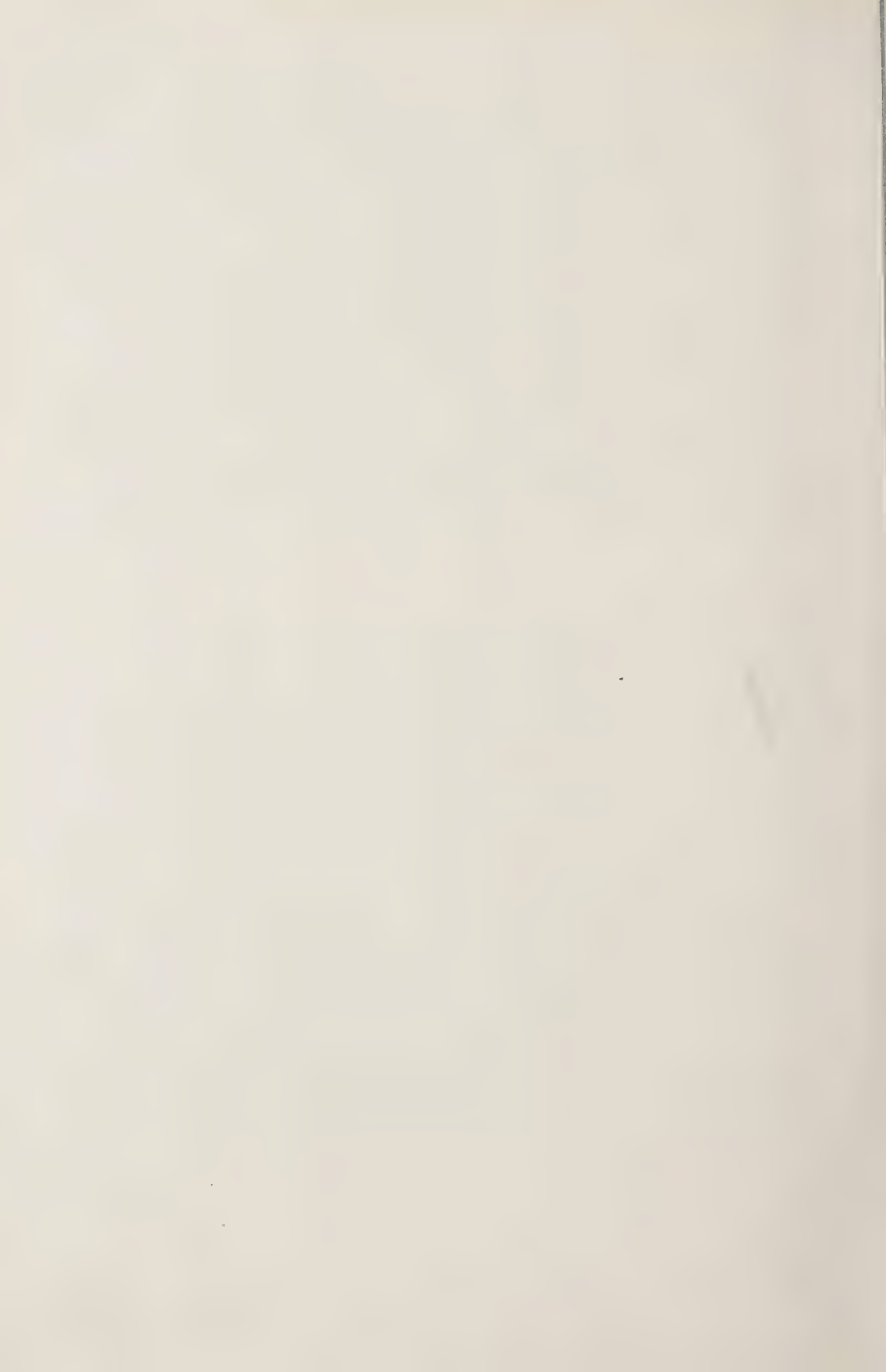




FIG. 1. *H. pseudospretella*. Eggs and young larvae.



FIG. 2. Bin of dried grass heavily infested by *H. pseudospretella* and *E. lactella*. Note mat of silk, frass and foodstuff, and, on sides, wandering larvae and cocoons of *H. pseudospretella*.



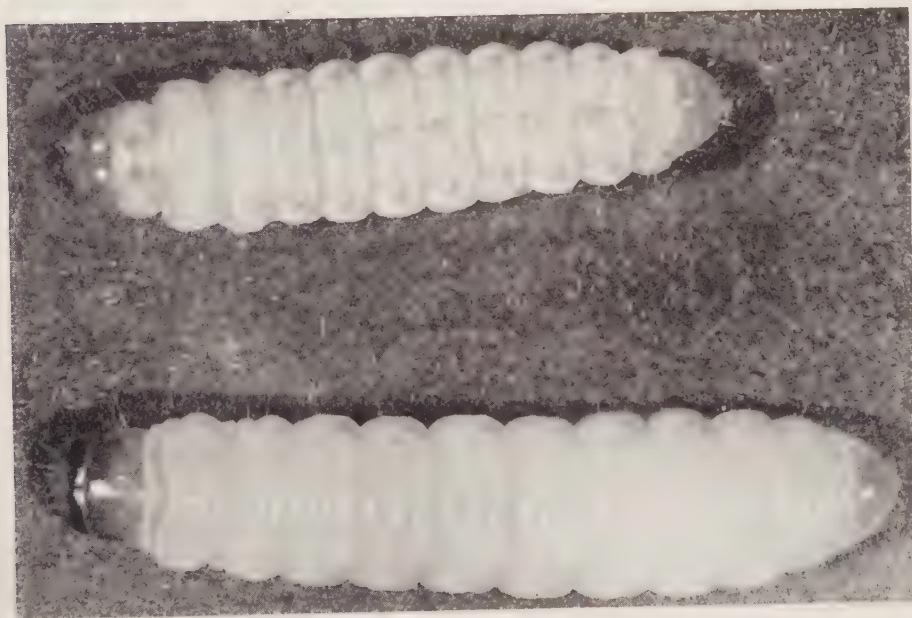


FIG. 1. *H. pseudospretella*. Wandering larva (left) and diapausing larva (right).

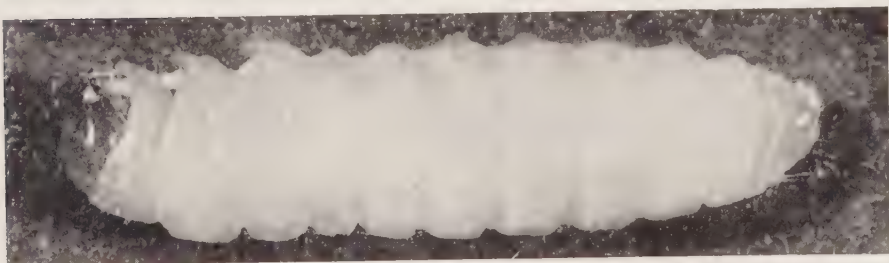


FIG. 2. *H. pseudospretella*. Prepupa.



FIG. 3. *H. pseudospretella*. Pupae.

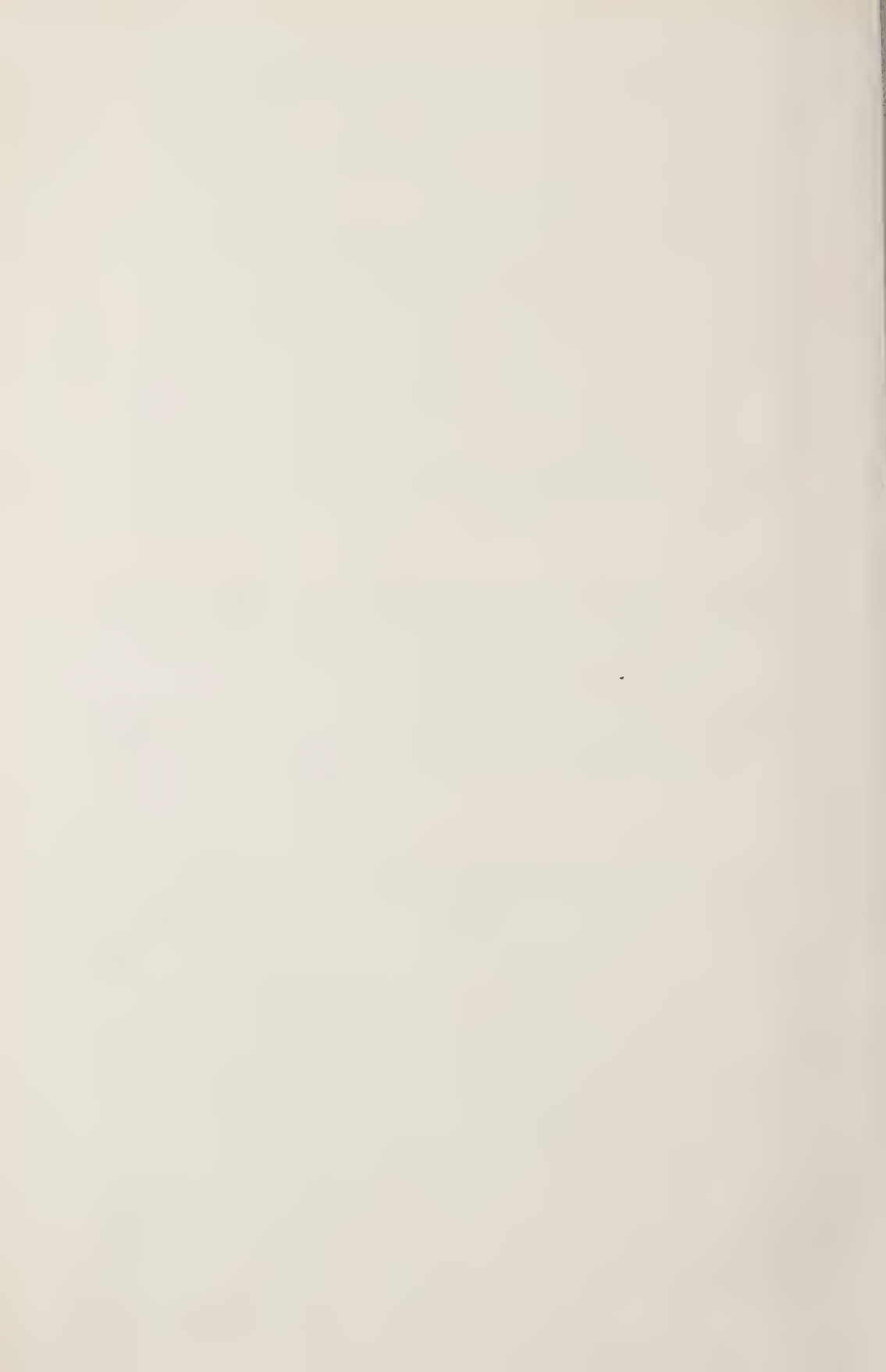
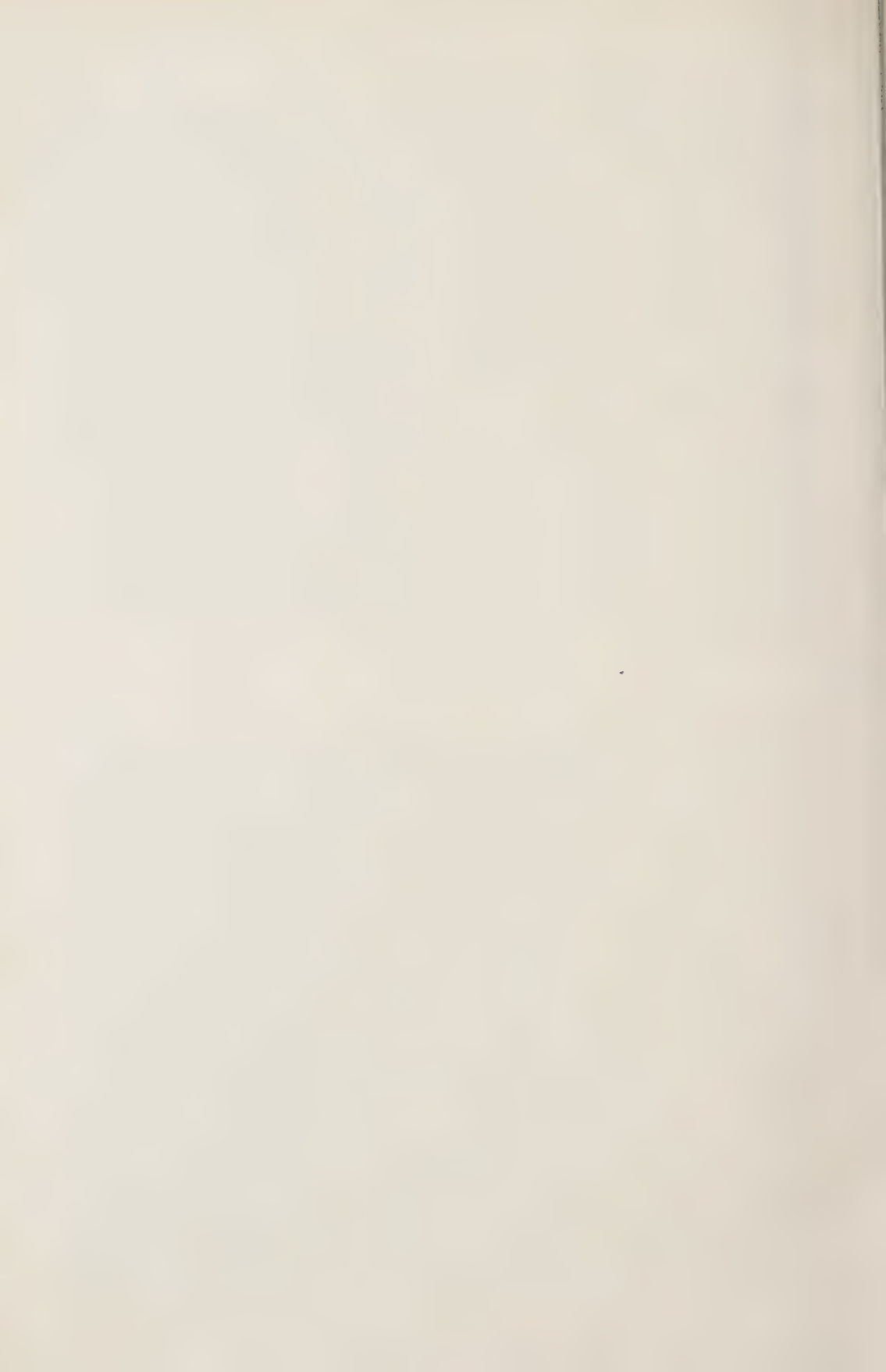




FIG. 1. *H. pseudospretella*. Adults. Female (left) and male (right).



FIG. 2. Copulation between *H. pseudospretella* (male) and *E. lactella* (female).



ACARICIDAL CONTROL OF THE TICK, *IXODES RICINUS* (L.) ON CATTLE.

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Considerable interest has been focussed on the control of various ticks in recent years, and the potentialities of different methods have been dealt with by a number of writers. Hitherto, the majority of control tests in Britain have been undertaken on sheep. With the exception of Milne's recent work (1948), little or no experimental work on the control of the sheep tick, *Ixodes ricinus* (L.), on pastures has been attempted.

During its free living phase, the sheep tick is found in, and on, matted vegetation of grass-land but in its parasitic phase, of course, it is attached to a host. Hence, it may be attacked in the pastures, or while feeding. In both phases the control measures aim at altering the normal ecological environment of the parasite. The use of acaricides alone on infested grass-land, or on stock, induces an immediate, but temporary change. Cultural methods readjust ecological plant succession, and thus modify conditions in the micro-habitat. Milne (*loc. cit.*) has, however, shown that a fairly considerable degree of cultural improvement is necessary to reduce, and maintain tick populations at a low level.

There are three effective methods of controlling *I. ricinus*, (a) eradication from hosts by means of acaricides, (b) pure cultural treatment of grass-land, and (c) using acaricides on grass-land, subsequent to cultural practices. Unfortunately effective pasture improvement is not an economic proposition on extensive hill and moorland grazings, such improvement as is possible can only supplement other control measures. On the contrary, a high proportion of the tick infested land in South Wales is confined to low-lying pastures (Arthur, 1948), with fenced boundaries, averaging 20 acres. Such pastures can be improved by the radical alteration of the parasites' habitat by ploughing, discing or pitch-poling, with or without subsequent treatment. In some of the infested pastures these treatments are not practicable on account of bad drainage or the difficult nature of the terrain. The logical approach in such cases is to eradicate the tick on the host and to prevent reinfestation. In the present paper experiments involving the use of acaricides in tick control on cattle will be described.

Control on Cattle.

A considerable amount of work on anti-tick treatment of cattle has been carried out abroad, in particular on dips containing arsenic. In general the number of cattle on any tick-infested farm in Britain is too small to justify the expenditure on this form of treatment, nor is the repetitive use of arsenic washes (much used abroad) considered to be a desirable practice with milking stock.

In Britain a number of workers (Hendrick & Moore, 1937; Moore, 1938, 1939; MacLeod, 1938, 1941, 1947; Stewart & Ponsford, 1939; Milne, 1945, 1948; and Heath & Mitchell, 1946, among others) have dealt with various aspects of the control of *I. ricinus* on sheep. Of these only Milne (1945) appears to have carried out experiments in relation to the pest on cattle, but no strictly quantitative data are available as the stock were not "handleable". The difficulties associated with the anti-tick treatment of cattle have been recognised, and much depends on the type of stock requiring treatment. Dairy cattle in South Wales give rise to most anxiety and it is with these that the writer is concerned.

Type of Acaricide desired.

The action of a wash, dip or spray may be toxic, repellent or antiseptic. The last mentioned merely inhibits bacterial action, and accordingly is not pertinent to the present problem. A repellent depends upon the odour of its volatile constituents to prevent attachment to the host. As dairy cattle spend the greater part of their lives on pastures, the volatile constituents are rapidly disseminated and the use of repellents would appear to be both wasteful and of limited duration as a preventative against reinfestation.

Toxic compounds may function through contact or ingestion. The first incision into the host skin by *I. ricinus* is generally made by the denticulate cheliceral digits (Sharif, 1928) and into this incision the hypostome and chelicerae are inserted. Blood is derived from the subepithelial tissues of the host, and feeding does not take place until the tick is firmly attached. Consequently ingestion effects are minimal and a contact acaricide would appear to be the most suitable. Ideally it should have the following properties : (i) a high rate of kill of attached ticks, irrespective of degree of engorgement ; (ii) a high residual toxicity ; (iii) be capable of retention on the host surface for a reasonable length of time, concomitant with practical difficulties, and possible ill-effects of repetitive treatment ; and (iv) be easy to prepare and economical in cost.

A number of chemicals have been employed for the destruction of *I. ricinus* on sheep, but this does not necessarily mean that the same chemicals would be effective on other hosts and tests on cattle are, therefore, necessary. The purpose for which the stock are kept influences the choice of chemicals. In the present experiments arsenic and coal-tar creosote washes have been avoided. The former because of the danger to stock and operators and of contamination of the milk ; the latter as tick reinfestation is rapid after such treatment (MacLeod, 1947) and the tainting of milk is a possibility.

Present Trials.

The trials were undertaken on dairy cattle on farms in the Margam district, South Wales. Only attached females were counted, as in the case of similar counts on sheep, the area selected being the hind quarters, including the groin and udder (Edwards & Arthur, 1947). The cattle were handwashed by the writer, who was also responsible for making the counts, so that variation due to personal error was minimised. The cattle were examined on the first, second and third days after treatment, and subsequently at three-day intervals. The tests were continued for 18-30 days to observe the rate of reinfestation.

The acaricides used were derris, DDT and pyrethrum, which are toxic to *I. ricinus* off the host. The problem hence resolves itself to (a) percentage mortality of attached ticks and (b) the degree of protection against reinfestation, introducing a time factor, measured in days for the period that a given preparation retains its toxicity. The period of time over which treatment continued to keep the adult infestation at less than 50 per cent. of the control is referred to as its effective duration. This does not represent the actual duration of protective effect for there is a "lag" period of recovery of an infestation brought to zero by the actual dipping or washing (MacLeod, 1947). In the absence of any protective effect the infestation will take some days to equal once more that of the undipped controls. Under constant conditions of exposure to infestation the period is about eight days for female ticks, since they usually feed for that time.

(i) *Derris*.—The wash contained 1 lb. derris powder (5.5 per cent. rotenone content), $\frac{1}{4}$ lb. soft soap and 1 gallon of water. The soft soap was dissolved in hot water, made up to 1 gallon with cold water, and poured onto the derris powder. A thick, dark brown fluid resulted, with derris particles in suspension. Preparation was carried on outside the cowshed to obviate the risk of blindness.

to the stock by airborne derris particles. Milne (private communication, 1.xi.1947) writes that there is "not much danger of blindness, unless a fairly large amount of powder reaches the eyes or wash runs into them". Unfortunately this suspension does not retain its toxicity, if kept for any length of time. Application to the skin was by means of a sponge soaked in the fluid.

(ii) *DDT* is effective in killing ticks under laboratory conditions (Burt, 1945), and retains its toxicity for a long time (Buxton, 1945). It is accordingly a potential anti-tick treatment. The original powder preparation contained 32 per cent. *DDT*; soft soap was employed as a wetting agent and the wash used at a dilution of 0.5 per cent. *DDT* applied by means of a sponge. As a dust *DDT* (5.0 per cent.) was mixed with pyrethrum (7.5 per cent.) and talc or Kaolin (87.5 per cent.) as a carrier.

(iii) *Pyrethrum* powder was kindly supplied by Stafford Allen and Sons Ltd. The total pyrethrum content was 0.9 per cent. (pyrethrins I and II), and it was used as a saponified aqueous wash.

Discussion of Results.

The results obtained are shown in figs. 1, 2, 3 and 4.

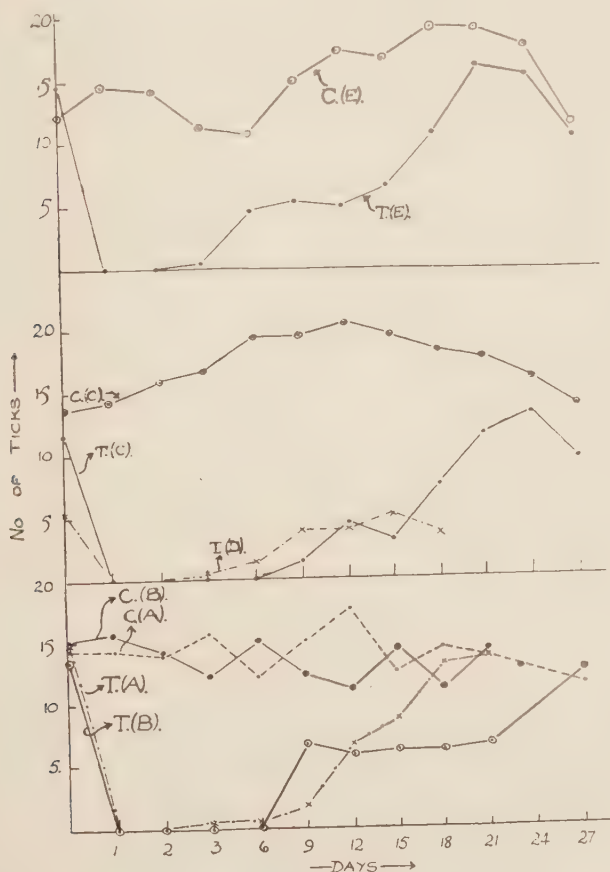


Fig. 1.—Tick counts on cattle. The effect of derris washes on tick populations.

Number of cattle washed with derris preparation 163.

Number of cattle used as controls 128.

T=treated cattle; C=control cattle. Capital letters in parenthesis refer to trials (abbreviations applicable also to figs. 2, 3 and 4).

The treatments had no adverse effect on the stock, which, on the contrary, seemed to thrive. This is presumably, due to the elimination of parasites (lice and warbles) other than ticks, and partly due to the tonic effects of a cold douche.

A presumed 100 per cent. mortality was noted within two days of derris wash treatment (fig. 1). Some of the ticks dropped off, while the remainder were attached as shrivelled remains. In trial A the tick population on the treated cattle exceeded 50 per cent. of the infestation of the controls on the 15th day; in B on 9th–12th days, and in trials C and E on 18th–21st days. The residual effects of the derris were thus apparent from nine to 21 days. In trial B heavy rain on the sixth day after treatment may have reduced the efficacy of the wash. The data, in general, bear out Milne's (1945) cruder observation.

Counts were made on washed and unwashed stock to determine the rate of individual tick pick-ups. Five milking cows were treated with the derris preparation and five untreated. Newly attached ticks were marked on their dorsal surfaces with a spot of quick-drying, green cellulose paint, and their positions mapped. Counts of freshly attached females were undertaken on 1, 2, 4, 5, 8, 10, 12, 15, 23, 25, and 27 days subsequent to washing. The results are shown in Table I. The rate of daily pick-up is independent of the original treatment; treated cows will pick up just as many ticks as untreated cows, because the parasites will grasp any moving object coming within their range. The two important points are (a) the number of ticks on the treated stock that succeed in attaching themselves, engorging and ovipositing successfully, (b) the duration of the period that the cattle remain tick free.

TABLE I.

Rate of female (*I. ricinus*) pick-up on derris treated and untreated cattle.

No. of days after treatment				No. of ticks picked up											
				Treated cattle						Untreated cattle					
				1	2	3	4	5	A _v	1	2	3	4	5	A _v
1	1	1	0	0	1	0.6	0	0	1	0	1	0.4
2	0	0	1	4	2	1.4	2	0	1	2	0	1.0
4	0	0	0	0	2	0.4	1	1	0	0	1	0.6
5	2	2	6	6	1	3.4	2	2	4	1	2	2.2
8	3	1	2	4	1	2.2	1	3	1	4	4	2.6
10	5	2	1	1	7	3.2	4	1	3	5	5	3.6
12	3	5	6	4	—	4.5	3	3	4	3	5	3.6
15	0	6	1	6	—	3.25	2	1	2	4	4	2.6
23	3	2	3	1	3	2.4	3	4	3	2	4	3.2
25	8	3	5	—	7	5.75	5	9	4	5	3	5.2
27	11	6	7	7	12	8.6	14	7	15	2	8	9.2

With the exception of the axillae, no engorging ticks were present until 12 days after washing. In the axillae, partially engorged females were present on the 8th–10th days. After the 12th day ticks, in various stages of repletion, were recovered from other parts of the body. A high proportion of these were, however, stupified or shrivelled from the effects of derris. Ticks, which fed to repletion, were removed from the cattle by the same method as employed elsewhere (Arthur, 1946), and laid eggs at the optimum temperature and humidity. Of 16 females recovered, nine laid less than 700 eggs apiece and six laid on the average 1,548, the lowest number from any one individual being 1,152. The females that attached themselves between the 18th and 27th days engorged, and laid an average number of 1,513 viable eggs.

It has been repeatedly observed that the axillae and groin are primary regions of reinfestation. Erosion of the derris particles is probably greater and more rapid in the groin, udder and angles of the front legs, on account of friction, accentuated by the fact that in the breeds examined they are areas of low hair density. Further, during the initial wash such areas are not likely to retain such a high concentration of the derris particles. The greater efficiency of derris on sheep is probably related to the nature of the coat. The majority of female ticks are located in the bare, hairy or short woolled parts, but Milne (1945) has shown that an appreciable infestation occurs in the long woolled areas. A greater quantity of derris (suspended in solution as ground root particles) is caught and retained by straining through the denser fleece, than by the hairy coat of cattle.

As in the case of derris, DDT (0.5 per cent.) wash became effective within 24 hours of application (fig. 2), and cattle remained free of ticks for three days in 21 cases out of 25. Analysing the data on an average basis, the cattle at centre F reached a 50 per cent. level of infestation in nine days, at G between 12th–15th days, and at H on 12th day. In trial F treated cattle attained populations approximately equal to the control on 18th day. Whilst DDT as a 0.5 per cent. wash may prove to be an efficient acaricide, the results obtained from the present field experiments do not allow for the formulation of specific recommendations. Similarly Heath and Mitchell (1946) obtained variable results with DDT in their sheep trials.

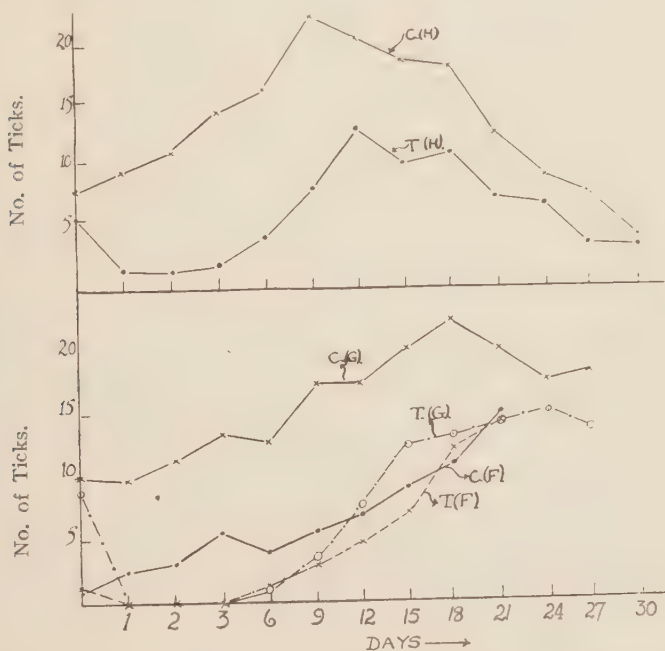


Fig. 2.—Tick counts on cattle. The effect of DDT washes on tick populations.
Number of cattle washed with DDT preparation 94.
Number of cattle used as controls 71.

DDT and pyrethrum powder mixture rubbed into the skin gave some immediate relief, but as a means of keeping cattle in a tick-free condition its value was low. Talc (I, J, K, fig. 3) was not a good carrier and was supplanted by Kaolin. At first

this mixture proved potent to attached ticks (L, M, N, fig. 3) but its residual toxicity was low. Where powder preparations were retained on the skin they crusted badly in patches.

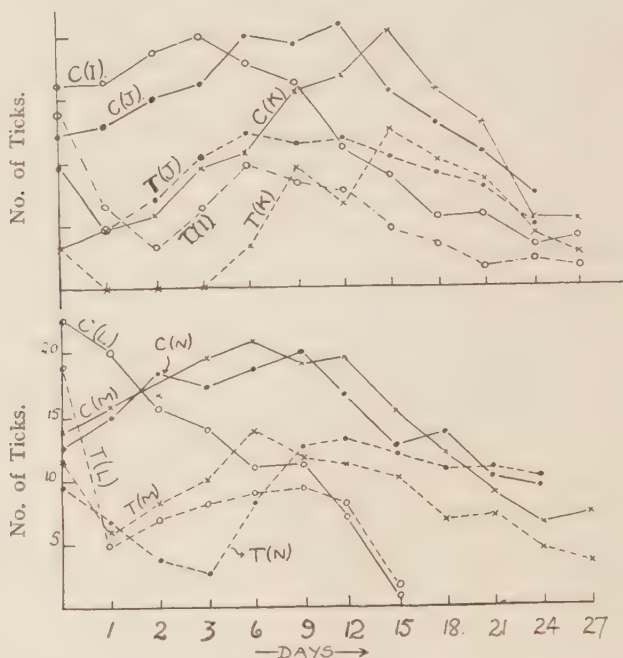


Fig. 3.—Tick counts on cattle. The effect of DDT-pyrethrum powder on tick populations.

Number of cattle powdered 123.

Number of cattle used as controls 60.

Aqueous suspensions of pyrethrum took a far greater toll of ticks that had not started to feed within 24 hours of washing. Those that remained were replete or nearly replete females and many of those partially engorged dropped from the host before maturity, while many of the replete individuals did not oviposit. When oviposition did take place (2 per cent. of the females) only a few viable eggs were deposited. The resistance of adult females seemed to be directly proportional to the degree of engorgement.

At centres O, P, and Q (fig. 4) reinfestation approached 50 per cent. of the control level between six and 12 days. Robinson (1944) has shown that pyrethrum is outstanding in its toxicity in both sprays and dusts against *Ornithodoros moubata* (Murray), but states that the powder used should be of a high quality, as it is likely to deteriorate rapidly in the field. Under these conditions the residual toxicity of pyrethrum would be much reduced.

In the summer the coats of cattle become thinner and in February–March natural moulting takes place (Craufurd-Benson, 1941). Tick activity commences in late March in South Wales (Edwards & Arthur, 1947), and anti-tick treatment in early March (to prevent reinfestation) is therefore likely to be ineffective as the reduction in hair density minimises the surface available to the derrick particles, and hairs, already treated, would fall out during moulting. After moulting, the skin of the cows becomes oilier, but as yet there is little or no evidence to show that this

materially affects the washes. It may, however, be of significance in the use of anti-tick dusts, when the secretion may act as a natural "sticker". Lyle Stewart (Stewart & Ponsford, 1939) used derris dusts on lambs with some success, and they attributed its residual toxicity to the higher concentration of derris retained in the fleece. Milne (1945) sprinkled cattle, tied in stalls, with derris-fuller's earth powder, rubbed in by hand. His results compared favourably with those on sheep. In the writer's experience three difficulties are associated with the use of powder preparations on cattle, (i) its inability to be retained on the skin for any length of time, especially in temperate oceanic climates, (ii) the high degree of waste during dusting, and (iii) the time taken in dusting is prohibitive for the stockman.

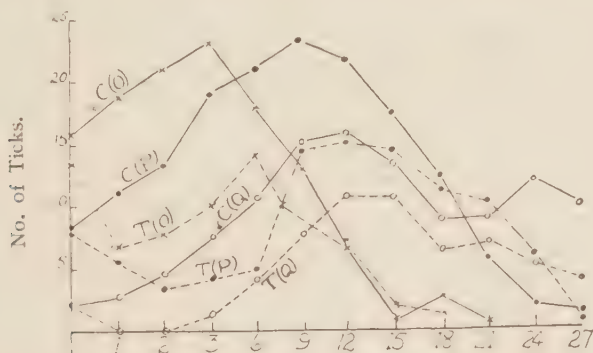


Fig. 4.—Tick counts on cattle. The effects of pyrethrum washes on tick populations. Number of cattle washed 39. Number of cattle used as controls 21.

The data presented show washes to be more efficient than powders in controlling ticks on cattle. With the exception of DDT-pyrethrum powders a satisfactory kill of attached females was apparent within 48 hours. They also indicate that variation occurs in the building up of infestations, and that reinfestation takes longer in cattle washed with derris preparations.

Summary.

Methods of approach to the control of *I. ricinus* by acaricidal action are outlined, and the type of acaricide for the control of ticks on dairy stock is discussed.

Dairy cattle were treated against ticks. Arsenic and carbolic washes were avoided for specified causes. The results of using (i) derris (5.5 per cent. rotenone), (ii) 0.5 per cent. DDT, (iii) DDT in a mixture with pyrethrum, talc or kaolin base, and (iv) pyrethrum (0.9 per cent. pyrethrins I and II) in aqueous solution show that (i) had a greater residual toxicity when applied to cattle, (ii) produced erratic results, which at present do not permit of specific recommendations. When DDT is employed in conjunction with pyrethrum as a powder (iii) the effective duration is low, and this is true also of pyrethrum as an aqueous wash (iv).

Acknowledgements.

Warmest thanks are due to the farmers of the Margam district, South Wales, for generous permission to use their cattle for the experiments, to Messrs. W. J. Bevan and T. W. Tyssul Jones for much assistance in the field and laboratory, to Dr. E. E. Edwards in whose Department the work was carried out, to Dr. John MacLeod, Research Fellow of the Veterinary Trust and Dr. Alec Milne, Agricultural Research Council, for much guidance and assistance in this work and for reading the manuscript.

References.

- ARTHUR, D. R. (1946). *Parasitology*, **37**, pp. 154-162.
- ARTHUR, D. R. (1948). *Bull. ent. Res.*, **39**, pp. 321-337.
- BURTT, E. T. (1945). *Ann. appl. Biol.*, **32**, pp. 247-260.
- BUXTON, P. A. (1945). *Trans. R. Soc. trop. Med. Hyg.*, **38**, pp. 367-400.
- CRAUFURD-BENSON, H. J. (1941). *Parasitology*, **33**, pp. 343-358.
- EDWARDS, E. E. & ARTHUR, D. R. (1947). *Parasitology*, **38**, pp. 72-85.
- HEATH, G. B. S. & MITCHELL, J. G. (1946). *Vet. J.*, **102**, pp. 130-140.
- HENDRICK, J. & MOORE, W. (1937). *Trans. Highl. agric. Soc. Scot.*, **49**, p. 158.
- MACLEOD, J. (1938). *Vet. Rec.*, **50**, pp. 1245-1250.
- MACLEOD, J. (1941). *Ann. appl. Biol.*, **28**, pp. 296-297.
- MACLEOD, J. (1947). *Ann. appl. Biol.*, **34**, pp. 207-223.
- MILNE, A. (1945). *Ann. appl. Biol.*, **32**, pp. 128-142.
- MILNE, A. (1948). *Ann. appl. Biol.*, **35**, pp. 369-378.
- MOORE, W. (1938). Report of the N.E. of Scotland Sheep Tick Committee. Aberdeen.
- MOORE, W. (1939). Second report of the N.E. of Scotland Sheep Tick Committee. Aberdeen.
- ROBINSON, G. G. (1944). *Bull. ent. Res.*, **35**, pp. 1-2.
- SHARIF, M. (1928). *Rec. Indian Mus.*, **30**, pp. 217-344.
- STEWART, W. L. & PONSFORD, A. P. (1939). *Vet. Rec.*, **51**, pp. 1481-1485.

THE SANDFLIES (PHLEBOTOMINAE) OF THE ANGLO-EGYPTIAN SUDAN.

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Our knowledge of the PHLEBOTOMINAE of the Sudan was brought up to date by Kirk and Lewis (1947) when 20 species and five varieties were listed, one of which, *Phlebotomus schwaetzi* var. *aethiopicus* Parrot, has now become a synonym. As a result of later surveys, made in connection with the study of leishmaniasis, the known range of these species has been extended and several others discovered, so that 36 species and eight varieties are now known to occur. The present paper is concerned mainly with distribution.

Sandflies in the Sudan are of practical importance in relation to kala-azar, oriental sore and sandfly fever, and on account of the irritation of their bites. Kirk (1939) described the epidemiology of leishmaniasis in the Sudan, and Kirk and Lewis (1947) referred to possible vectors of which *P. orientalis* Parrot, appears to be the principal one in the eastern and western areas. *P. papatasi* (Scop.), which is unlikely to be concerned in the transmission of kala-azar (Adler, 1947) is the common biting species in houses and is presumed to be the vector of sandfly fever.

Methods.

Collecting.

Oiled paper traps were used, as previously described (Kirk & Lewis, 1940).

Mounting.

Staining and mounting thousands of specimens in canada balsam would have been impossible in the time available. After several media had been tried Puri's was adopted for general use. It was prepared as described by Hopkins (1936), except that the liquid was filtered through a small wad of cotton wool at the bottom of a glass funnel standing in a tin heated by the sun to reduce viscosity. The use of cotton wool instead of layers of muslin reduced wastage due to absorption. Canada balsam was found to be unsatisfactory for ringing because often it eventually penetrated the medium. Cover glasses $\frac{3}{8}$ in. in diameter were used and ringed with Puri's medium itself. Further attention was seldom necessary.

Classification.

Kirk and Lewis (1946a) recognised three subgenera of *Phlebotomus* in Africa: *Phlebotomus* (s. str.) Rondani, 1840, *Sintonius* Nitzulescu, 1931, and *Prophlebotomus* França & Parrot, 1921. Theodor (1948) divides the Old World PHLEBOTOMINAE into two genera, *Phlebotomus* and *Sergentomyia*, with 12 subgenera and several groups. Although it may be of great assistance in the determination of species, this classification requires much alteration of nomenclature and is in some respects artificial. Several of the subgenera might be regarded instead as groups (*Larroussius*, *Adlerius*, *Euphlebotomus*), and certain of the groups (that of *P. squamipleuris*, for example) as subgenera. For these reasons we have decided to retain, in the present stage of knowledge, the simpler classification of Kirk and Lewis mentioned above and the species are arranged here in the order of Theodor's paper, with the exception of *P. cinctus* and *P. occidentalis*. The Sudan species may be

identified by means of the keys of Kirk and Lewis (1946b, 1948) and by reference to original descriptions.

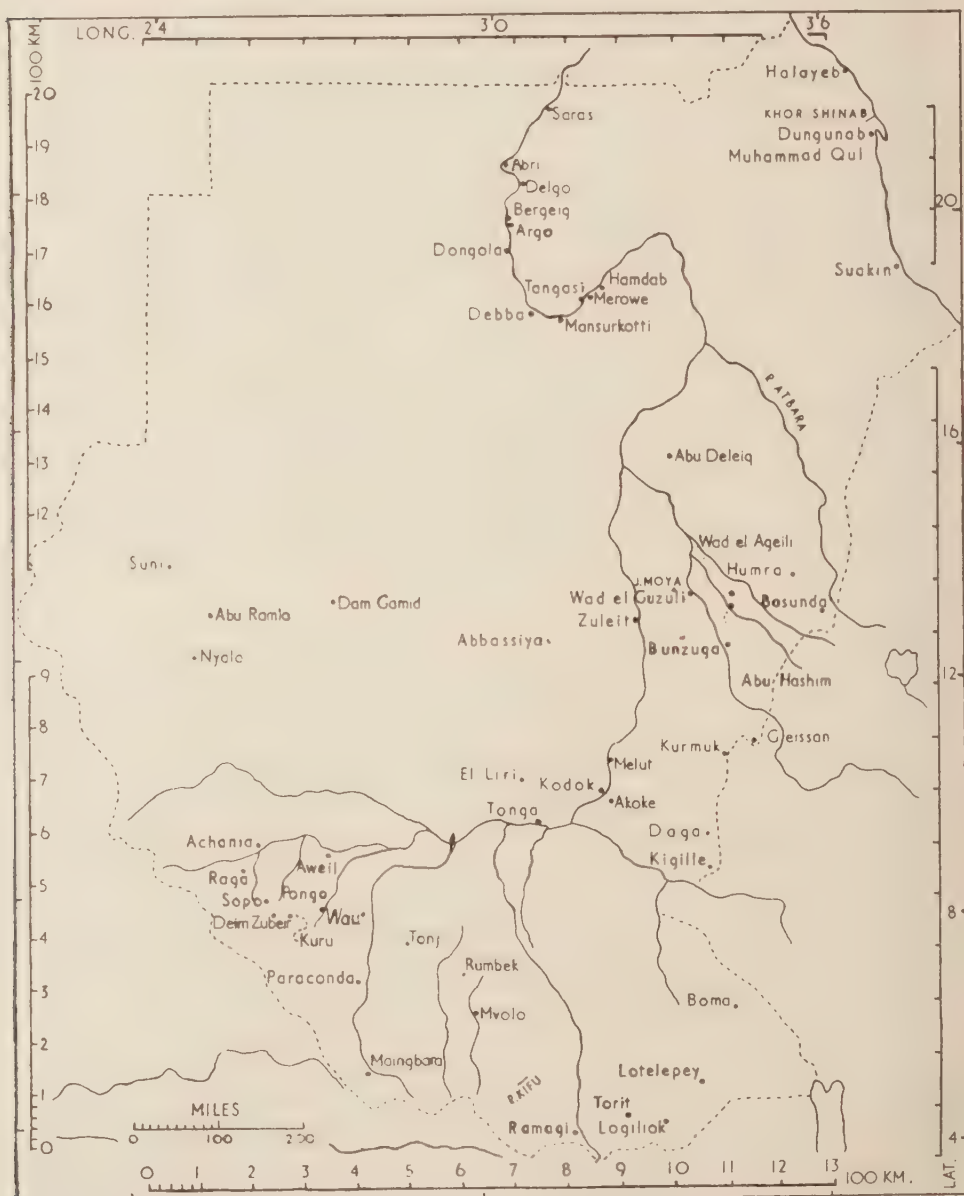


Fig. 1.—The Sudan showing places mentioned in the text and not shown in previous papers.

The Species in the Sudan.

The localities in the following paragraphs are additional to those recorded by Kirk and Lewis (1940, 1947). Akobo, Diner and Hamra in previous papers should

be written Akoke, Dinder and Humra. All records are shown in figs. 2 to 17, except a few old ones, which are now considered invalid, and some places close together.

Phlebotomus (Phlebotomus) papatasi (Scop.).

This species has been recorded from Debba, Delgo, Dongola, Hamdab, Humra, Malakal, Mansorkotti, Merowe, Saras, Sennar, Khor Shinab, Suakin and Tonga.



Fig. 2.—*P. papatasi*, *P. p.* var. *bergeroti* and *P. roubaudi*.

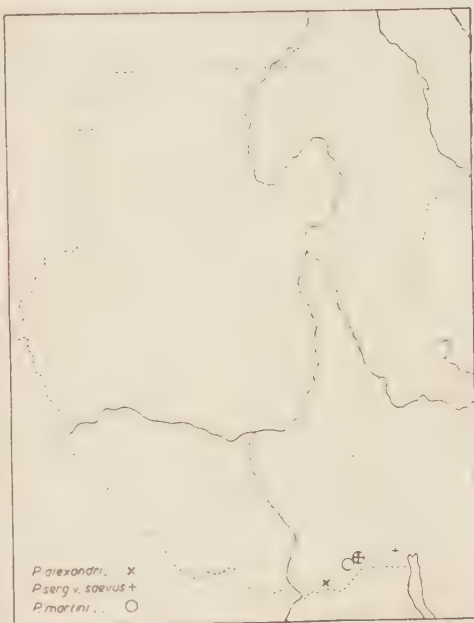


Fig. 3.—*P. alexandri*, *P. sergenti* var. *saevus* and *P. martini*.

After the high Nile flood of 1946, much riverain land was covered with cracking silt which was regarded as the source of outbreaks of *P. papatasi* at Berber, Delgo, Wadi Halfa and elsewhere. In the Gezira irrigated area this species is said to become troublesome each year when the cotton stalks are pulled up and burnt, which is about six weeks after irrigation ceases and not long after the beginning of the hot season. The insects have probably had time to breed in the cracks which form in the drying soil as well as in cracks in fallow ground.

Sandfly fever, of which this sandfly is presumed to be the vector, is not necessarily associated with large numbers of the insects but occurs in the form of occasional epidemics. The Sudan Medical Service Annual Reports record two outbreaks in Khartoum, one apparently associated with building operations and another during which no increase of sandflies was noted.

Phlebotomus (Phlebotomus) papatasi var. *bergeroti* Parrot.

The one new record is from Suakin.

Phlebotomus (Phlebotomus) roubaudi Newstead.

There are no additional records of this or the following four species :—

Phlebotomus (Phlebotomus) alexandri Sinton.

Phlebotomus (Phlebotomus) sergenti var. *saevus* Parrot & Martin.

Phlebotomus (Phlebotomus) martini Parrot.

Phlebotomus (Phlebotomus) longipes Parrot & Martin.

Phlebotomus (Phlebotomus) orientalis Parrot.

The one new record is from Wad el Ageili.

Phlebotomus (Phlebotomus) lesleyae Lewis & Kirk.

This species has been found at Kortala (Lewis & Kirk, 1946a), Kosti and Wad Medani.

Phlebotomus (Phlebotomus) rodhaini Parrot.

New records are from Hawata, Kuru, Mvolo and R. Zeraf (km. 121).

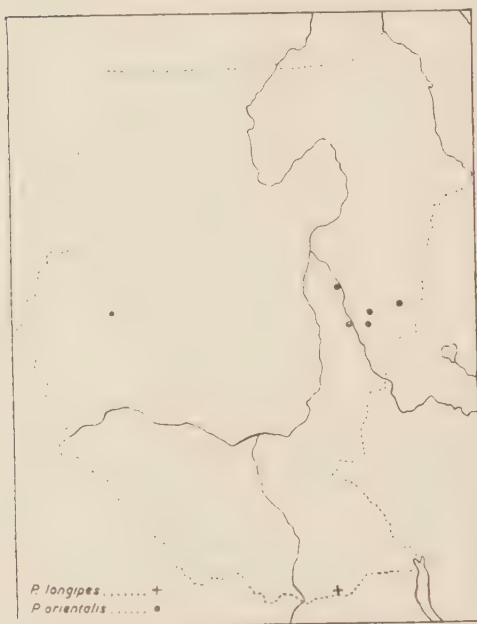


Fig. 4.—*P. longipes* and *P. orientalis*.

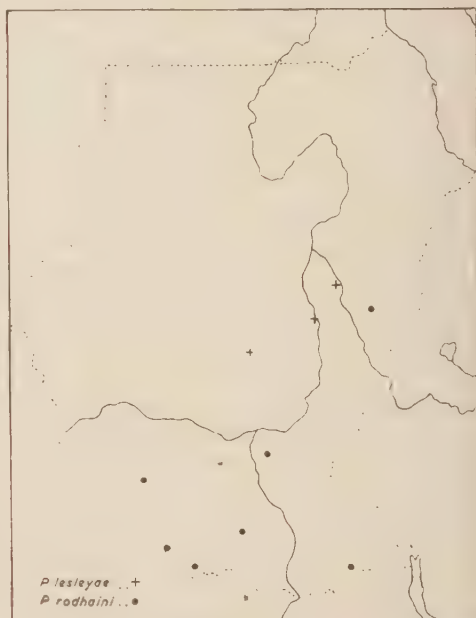


Fig. 5.—*P. lesleyae* and *P. rodhaini*.

Phlebotomus (Prophlebotomus) buxtoni Theodor.

This species has only been found at Wau (Parrot, 1948b).

Phlebotomus (Prophlebotomus) congolensis Bequaert & Walravens.

P.c. var. *distinctus* is commoner than the type form. The two are shown as one in fig. 6, additional records being from Boma, Bunzuga, Debba, Deim Zubeir, Dongola, Geissan, Kadugli, Keilak, Kigille, Kortala, Kurmuk, Kuru, Launi, El Liri, Lotelepey, Maingbara, Jebel Moya, Mvolo, Raga, Roseires, Sennar, Sopo, Sources Yubu, Suni, Tonj and Wau.

Phlebotomus (Prophlebotomus) congolensis var. *distinctus* Theodor.

The distribution is given with that of the type form.

Phlebotomus (Prophlebotomus) cowlandi Lewis & Kirk.

Theodor (1948) considers that this species is probably identical with *P. congolensis*. It is known only from the type locality, Gallabat (Lewis & Kirk, 1946a).

Phlebotomus (Prophlebotomus) schoutedeni var. *pungens* Parrot.

This sandfly is known only from the type locality, Li Rangu (Parrot, 1948a).



Fig. 6.—*P. buxtoni*, *P. congolensis*, *P. c.* var. *distinctus*, *P. cowlandi* and *P. schoutedeni* var. *pungens*.

P. schout. v. vorax in fig. 6 should read *P. schout. v. pungens*.—Ed.



Fig. 7.—*P. signatipennis*, *P. cinctus* and *P. occidentalis*.

Phlebotomus (*Prophlebotomus*) *signatipennis* Newstead.

P. cinctus and *P. occidentalis* are very similar to *P. signatipennis* and may eventually be regarded as varieties of it (Parrot, 1948b). All three are treated as one in fig. 7.

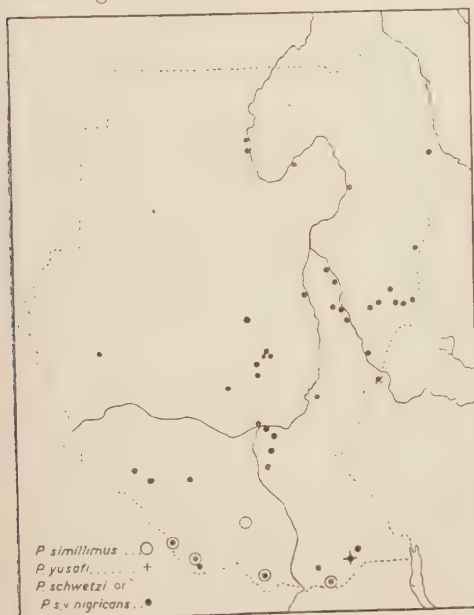


Fig. 8.—*P. simillimus*, *P. yusafi*, *P. schwetzi* and *P. s.* var. *nigricans*.



Fig. 9.—*P. africanus*, *P. a.* var. *eremitis*, *P. a.* var. *niger* and *P. a.* var. *sudanicus*.

South of about latitude 12° most females have a rather small pharyngeal armature and are probably *P. cinctus*.

Additional records of this species are from Abbassiya, Abu Deleig, Abu Hashim, Achania, Akoke, Amadi, Argo, Atbara, Aweil, Bunzuga, Debba, Deim Zubeir, Dinder, Doka, Dongola, Dungunab, Fanjak, Halayeb, Humra, Jebel Moya, Kassala, R. Kipu area, Kodok, Kurmuk, Kuru, Li Rangu, Logiliok, Lul, Melut, Merowe, Muhammad Qul, Mvolo, Nyala, Paraconda, Pongo, Raga, Rashad, Rumbek, Saras, Khor Shinab, Suakin, Tonga, Torit and Wau.



Fig. 10.—*P. ingreni*, *P. kirki* and *P. serratus*.

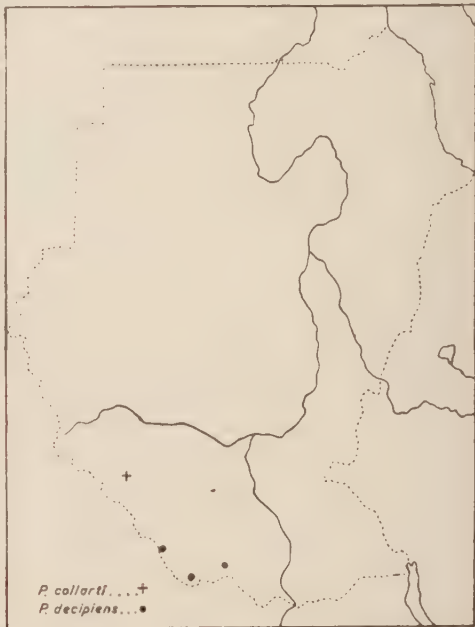


Fig. 11.—*P. collarti* and *P. decipiens*.

P. signatipennis or its two allies have been found biting three species of lizards, *Tarentola annularis* Geoff. at Wad Medani (three times), *Hemidactylus brooki* Gray at Heiban, and *H. turcicus* (L.) at Akoke.

Phlebotomus (*Prophlebotomus*) *cinctus* Parrot & Martin.

This species occurs at Gedaref, Kuru, Launi, Lotelepey and Wau (Parrot, 1948b), and at many other places. Although structurally much like *P. signatipennis* and possibly a variety of it, *P. cinctus* differs considerably in its distribution, being almost restricted to the southern half of the country.

Phlebotomus (*Prophlebotomus*) *occidentalis* Theodor.

This species occurs at Paraconda (Parrot, 1948b).

Phlebotomus (*Prophlebotomus*) *simillimus* Newstead.

New records are from Li Rangu, Mvolo and Yei.

Phlebotomus (*Prophlebotomus*) *yusafi* Sinton.

This species has been found at Kapoeta.

Phlebotomus (Prophlebotomus) schwetzi Adler, Theodor & Parrot.

New records, including a few for var. *nigricans*, are from Argo, Doka, Dongola, Erkowit, Geissan, Hamdab, Heiban, Humra, Kuru, Lotelepey, Jebel Moya, Nyala, Sopo, Torit, Wau and R. Zeraf (km. 121).

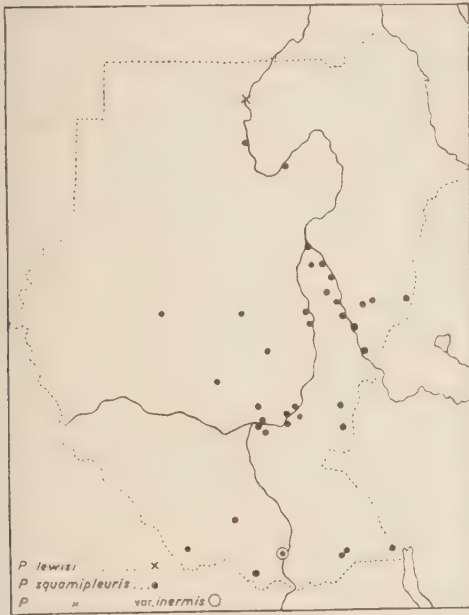


Fig. 12.—*P. lewisi*, *P. squamipleuris* and *P. s. var. inermis*.

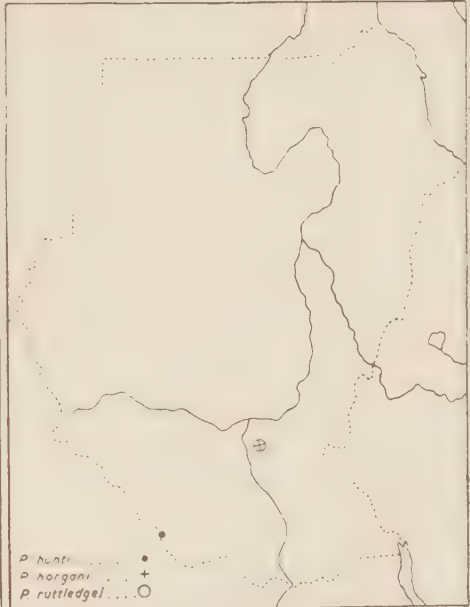


Fig. 13.—*P. hunti*, *P. horgani* and *P. rutledgei*.

Phlebotomus (Prophlebotomus) schwetzi var. *nigricans* Parrot.

This variety was described from a Katire specimen by Parrot (1948b).

Phlebotomus (Prophlebotomus) africanus Newstead.

Additional records for *P. africanus* or var. *sudanicus* are from Agur, Abbassiya, Abu Ramla, Akoke, Aweil, Basunda, Bunzuga, Deim Zubeir, Delami, Erkowit, Humra, Kadugli, Katcha, Khartoum, R. Kipu area, Kosti, Kurmuk, Launi, Li Rangu, El Liri, Manaquil, Jebel Moya, Mvolo, Nyala, El Obeid, Ramagi, Rumbek, Roseires, Suakin, Tokar, Tonj, Umm Ber, Wad el Ageili, Wau and Yei.

Phlebotomus (Prophlebotomus) africanus var. *eremitis* Parrot & de Jolivière.

This variety has been found at Debba (Parrot, 1948b).

Phlebotomus (Prophlebotomus) africanus var. *niger* Parrot & Schwetz.

The one new record is from Mvolo.

Phlebotomus (Prophlebotomus) africanus var. *sudanicus* Theodor.

The distribution of this variety is shown with that of the type form.

Phlebotomus (Prophlebotomus) ingrami Newstead.

Additional records are from Li Rangu and Ramagi.



Fig. 14.—*P. adleri*, *P. affinis* and *P. a.*
var. *vorax*.

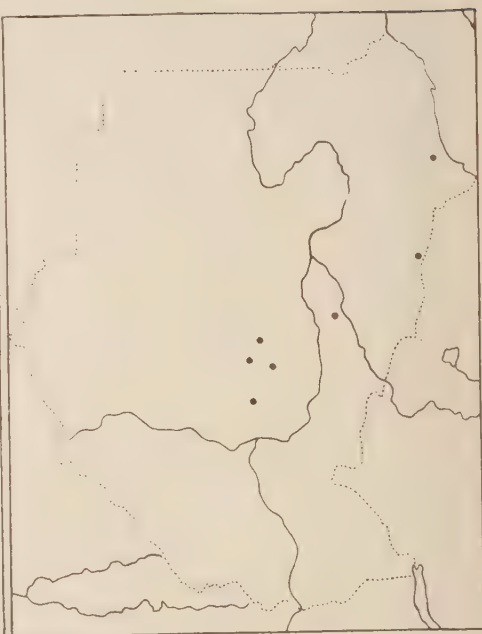


Fig. 15.—*P. calcaratus*.

Phlebotomus (*Prophlebotomus*) *kirki* Parrot.

This was described from Li Rangu specimens by Parrot (1948b).

Phlebotomus (*Prophlebotomus*) *serratus* Parrot & Malbrant.

This species occurs at Gilo (Parrot, 1948a) and Yambio.

Phlebotomus (*Prophlebotomus*) *collarti* Adler, Theodor & Parrot.

One male has been found at Sopo.

Phlebotomus (*Prophlebotomus*) *decipiens* Theodor.

There are no additional records.

Phlebotomus (*Prophlebotomus*) *lewisi* Parrot.

The type female was taken at Abri near Wadi Halfa (Parrot, 1948a).

Phlebotomus (*Prophlebotomus*) *squamipleuris* Newstead.

This species has been recorded from Akeke, Daga, Dam Gamid, Dongola (Parrot, 1948b), Hawata, Kodok, Maingbara, Mvolo, El Obeid, Roseires, Sennar, Tangasi, Wad Arud and Zuleita.

Males can be quickly recognised under a low magnification by the large size of the slightly tapering sixth abdominal segment, combined with a dark colouring which distinguishes them from those of *Sintonius*.

In addition to differing structurally from nearly all other sandflies, *P. squamipleuris* shows unusual biological features. It is associated with damp conditions and is attracted to artificial light more than most species. Adults have often been taken on the banks of rivers and at Wad Medani they are common in the rainy season when other species diminish. On one occasion in August at 10 p.m.

females were found feeding on the heads of frogs, probably *Rana* (*Ptychadena*) *floweri*, which were half submerged in a pool of rain water. Other *P. squamipleuris* were floating on the water or standing on wet mud.

In Table I of Kirk and Lewis (1947) the figures for sandflies attracted to light should be 72 *P. squamipleuris* and 27 *P. signatipennis*.

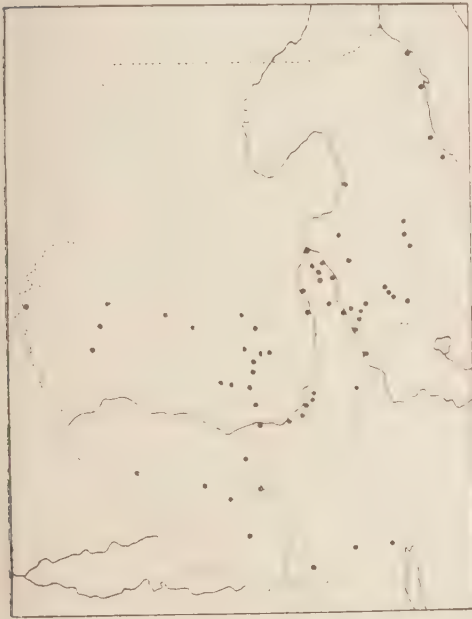


Fig. 16.—*P. clydei*.

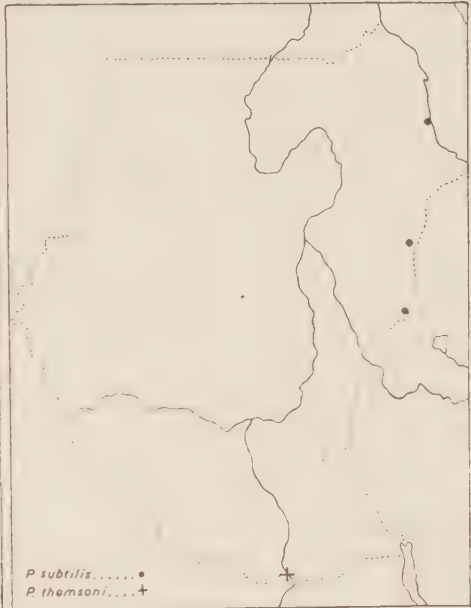


Fig. 17.—*P. subtilis* and *P. thomsoni*.

Phlebotomus (*Prophlebotomus*) *squamipleuris* var. *inermis* Theodor.

A single female has been found at Juba.

Phlebotomus (*Prophlebotomus*) *hunti* Lewis & Kirk.

There are no additional records of this or the following two species.

Phlebotomus (*Prophlebotomus*) *horgani* Lewis & Kirk.

Theodor (1948) considers that this species may be identical with *P. wurtzi* Parrot.

Phlebotomus (*Prophlebotomus*) *ruttledgei* Lewis & Kirk.

Phlebotomus (*Sintonius*) *adleri* Theodor.

This species and *P. affinis* have been found chiefly on or near rocky areas. New records are from Jebel Moya and Kadugli. A male, apparently of this species, has been found at Bergeig.

Phlebotomus (*Sintonius*) *affinis* Theodor.

The male shows no significant difference from that of *P. a.* var. *vorax*. Specimens of *P. affinis* from the Nuba Hills were very pale.

Distribution: The type form is known to occur at Agur, Jebel Moya, Kadugli, Li Rangu (Parrot, 1948a), El Liri, Mvolo, Rashad and Wau. The type locality in Equatoria Province is unknown but is thought to be in the Kapoeta area.

Phlebotomus (Sintonius) affinis var. *vorax* Parrot.

This variety is only known from El Fasher (Parrot, 1948a).

Phlebotomus (Sintonius) calcaratus Parrot.

Some male specimens have buccal teeth rather close together so that they can resemble *P. clydei*, but *P. calcaratus* is easily distinguished from this species by the shape of the pharynx, the great width of the posterior part of the buccal cavity, and the presence of femoral spines. In some mounted specimens the buccal teeth are curved downward and forward so that they have a nodular appearance. Very pale specimens have been found in the Nuba Hills.

Distribution: *P. calcaratus* is usually taken on or near rocky hills, and has been found at Erkowit, Jebel Moya (type male, Parrot, 1948a), Kassala (type female), Kortala, Rahad, Rashad and Talodi.

Phlebotomus (Sintonius) clydei Sinton.

Additional records are from Abu Deleig, Abu Hashim, Abu Ramla, Amadi, Atbara, Bunzuga, Dam Gamid, Deim Zubeir, Dinder, Gedaref, Halayeb, Khartoum, Kurmuk, Jebel Moya, Muhammad Qul, Nahud, Nyala, Rashad, Rumbek, Suakin, Tonj, Torit, Wad el Ageili and Wad el Gazuli.

Phlebotomus (Sintonius) subtilis Parrot.

This species occurs at Gallabat (Parrot, 1948b), Kassala and Port Sudan.

Phlebotomus (Sintonius) thomsoni Theodor.

This species has been found at Kajo Kaji.

General Remarks on Distribution.

The Sudan is situated almost entirely within the Ethiopian zoogeographical region and near its north-east corner. Its sandfly fauna is composed mainly of Ethiopian species but has Palaearctic and Oriental affinities. *P. papatasi* (which is not found far south) and *P. alexandri* are largely Palaearctic in distribution; *P. africanus* var. *cremitis* is evidently an intruder from the Sahara; and *P. papatasi* var. *bergeroti*, *P. roubaudi*, *P. sergenti* var. *saevus* and *P. subtilis* are closely allied to Palaearctic forms. *P. clydei* has an extensive Oriental distribution; *P. calcaratus* is allied to the Oriental *P. christophersi*, and *P. kirki* to the Oriental *P. zeylanicus* and *P. sylvestris* (Parrot, 1948a).

A relatively small area in the south-west forms part of the West African Sub-region, as shown in Map 2 of Lewis (1947) and by Edwards (1941, p. 452), and contains several sandflies, *P. schoutedeni* var. *pungens*, *P. simillimus*, *P. africanus* var. *niger*, *P. ingrami*, *P. kirki*, *P. serratus*, *P. decipiens* and *P. huntii*, which are rare or absent further north.

With regard to the East and South African Sub-region, the small Sudan section of the Eastern and Southern Province is the only part of the country where *P. sergenti* var. *saevus* and *P. longipes* are found. The whole of the Sudan coastal area may perhaps be regarded as part of the Somali Arid District and contains *P. subtilis*, a species which was described from Ethiopia and one of us has found commonly in Eritrea. Both *P. subtilis* and *P. papatasi* var. *bergeroti*, together with some coastal mosquitos, occur at Kassala which should perhaps be regarded as part of the Somali Arid District.

Most of the Sudan forms part of the Sudanese zoogeographical Province and the distribution of its sandflies shows a striking contrast to that of the mosquitos which have been discussed by Edwards (1941). He found that there appeared to be no clear line of demarcation between the Guinean and Sudanese Savanna Provinces,

and that the Sudanese Arid District supported very few species, some of which were mainly Palaearctic. Reference to the maps in the present paper shows that the sandflies of the Ubangi-Uelle Savanna District scarcely extend into the Sudanese Savanna and that conversely several northern species are scarce or absent in the extreme south-west. Also many sandflies, with the notable exception of *P. cinctus*, extend from the Sudanese Savanna well into the Sudanese Arid District. Like the mosquitos, the sandfly fauna includes several species, particularly *P. papatasi*, which have Palaearctic affinities and occur in the north.

The Kapoeta area is the only part of the country where *P. roubaudi*, *P. alexandri*, *P. martini* and *P. yusafi* have been found. It may be regarded as a distinct ecological area, more arid than the rest of the southern Sudan, and has been shown to have a different, northern, type of vegetation by Tothill (1948, p. 34). The presence of *P. alexandri* so far beyond its normal range (Lewis & Kirk, 1949) is difficult to understand.

Of the widely distributed sandflies *P. papatasi*, *P. signatipennis*, *P. cinctus*, *P. africanus* and *P. clydei* are very common, and *P. congolensis* var. *distinctus*, *P. schwetzi* and *P. squamipleuris* are common. It is surprising that such small delicate insects with a weak flight should be so widely distributed, but their association with soil fissures (referred to below) provides them with an extensive environment and leaves them almost unaffected by day-time atmospheric conditions.

Among the common species one may note the absence or rarity of *P. signatipennis* and its two allies, and of *P. clydei*, from the far south-west, the absence of *P. africanus* and *P. clydei* along the northern part of the Nile and the absence of *P. squamipleuris* in the coastal area.

The survey of the less common species takes a long time owing to the necessity of mounting and examining many of the common ones, so some of the less common species may in time prove to be more widespread.

The Fissure Environment.

Distribution.

The fissures which develop in cracking clay during the dry season constitute a vast subterranean environment for sandflies and several other animals (Kirk & Lewis, 1947). The districts in which sandflies are most abundant are determined by the conditions which produce deep cracks, namely, a clay subsoil which is saturated each year with rain or river water and then left to dry for several months. The subsoil, rainfall and topography of the Sudan are shown by Tothill (1948, pp. 34, 69, 84, 88 and 137). The main area of cracking clay lies partly between the White Nile and Atbara rivers, and follows the White Nile southwards, spreading out in the region of the great swamps, and including the above-mentioned Kapoeta area. The distribution of rainfall is such that most of the clay area has a dry season long enough to allow extensive cracking, but it is divided into east and south-eastern sections by an extensive area in the centre which is flooded by river water for many months each year. The eastern and south-eastern areas are important kala-azar regions of the Sudan.

Bats and soil fissures.

In the Dueim area, at least, bats use the fissure environment and at dusk large numbers of them can be seen rising from the cracks with their wings lit up by the setting sun. At Wad Medani bats are said to be commonest inland during the rains when the rising river fills the cracks along its banks. Sandflies have not been seen to feed on the bats but are common at certain roosting sites, and Wanson (1942) has identified bats' blood in sandflies.

Control.

Control of breeding.

In the northern Sudan soil fissures are believed to be the main breeding places of sandflies, and cracks in the walls of houses are probably too dry for the larvae. The soil fissures are often automatically filled or covered by irrigation of gardens, seasonal rains, and the trampling of people and animals. It is often noticed that houses on the periphery of a town, near the untrodden cracked soil, are more infested by *P. papatasi*.

Destruction of adults.

It is known that DDT has a residual effect on *P. papatasi* and in the Sudan favourable reports on the reduction of sandflies have been received after the treatment of houses.

We found it impossible to keep *P. papatasi* and *P. clydei* alive in captivity for more than a very few days in an area where DDT was being used, but as soon as the insects, tubes and other equipment were moved three miles away and kept under similar atmospheric conditions the early mortality ceased. The trouble was attributed to DDT blown about among the dust particles in the air.

Personal protection.

Sleeping on the roofs of bungalows does not always afford immunity from the bites of *P. papatasi*, but we have found dimethyl phthalate to give protection against this species for several hours. This repellent is marketed by the Sudan Medical Service at a controlled price as a public health measure against various insect-borne diseases and should give considerable protection against kala-azar to persons visiting infected areas.

Summary.

This paper is mainly an account of the known distribution of the 36 species and eight varieties of PHLEBOTOMINAE found in the Sudan. Their economic importance is briefly discussed.

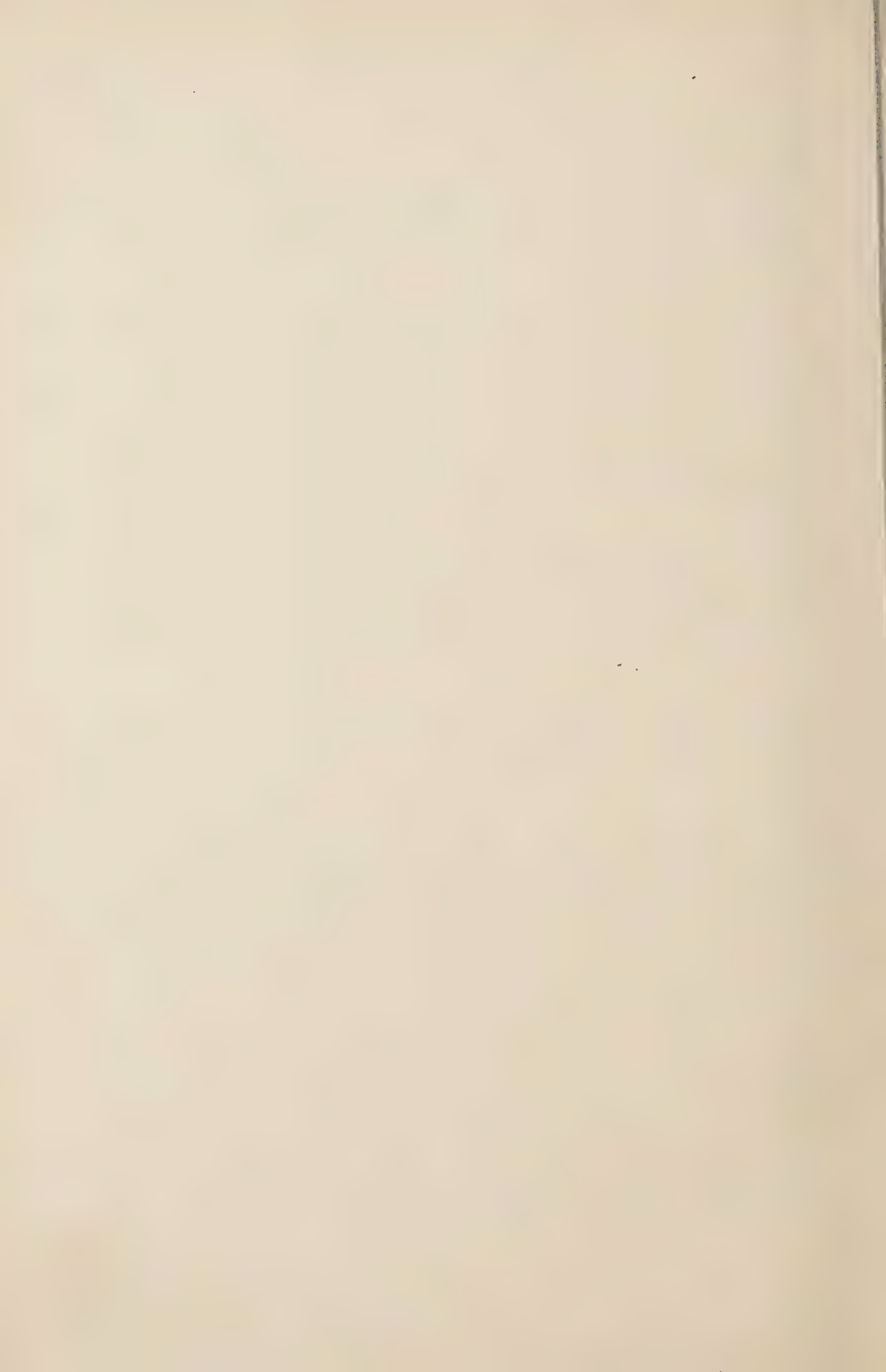
Acknowledgements.

We are much indebted to the Director of the British Museum (Natural History) for the identification of the vertebrates mentioned, and to Dr. P. H. Abbott, Dr. J. F. E. Bloss, Tewfik Eff. A. Khalifa, Muhammad Eff. A. Muhammad and Mr. C. E. Wilson, who have kindly sent us sandflies for identification from the Suni, Daga, Abu Hashim, Halayeb and Kurmuk areas respectively.

References.

- ADLER, S. (1947). The behaviour of a Sudan strain of *Leishmania donovani* in *Phlebotomus papatasi*. A comparison of strains of *Leishmania*.—Trans. R. Soc. trop. Med. Hyg., **40**, pp. 701-712.
- EDWARDS, F. W. (1941). Mosquitoes of the Ethiopian Region III. Culicine adults and pupae.—499 pp. London, Brit. Mus. (Nat. Hist.).
- HOPKINS, G. H. E. (1936). Mosquitoes of the Ethiopian Region I. Larval bionomics of mosquitoes and taxonomy of Culicine larvae. 250 pp. London, Brit. Mus. (Nat. Hist.).
- KIRK, R. (1939). Studies in leishmaniasis in the Anglo-Egyptian Sudan. Part I. Epidemiology and general considerations.—Trans. R. Soc. trop. Med. Hyg., **32**, pp. 533-544.

- KIRK, R. & LEWIS, D. J. (1940). Studies in leishmaniasis in the Anglo-Egyptian Sudan. III. *Ibid.*, **33**, pp. 623-634.
- KIRK, R. & LEWIS, D. J. (1946a). Taxonomy of the Ethiopian sandflies (*Phlebotomus*). I. Classification and synonymy.—*Ann. trop. Med. Parasit.*, **40**, pp. 34-51.
- KIRK, R. & LEWIS, D. J. (1946b). Taxonomy of the Ethiopian sandflies (*Phlebotomus*). II. Keys for the identification of the Ethiopian species.—*Ibid.*, **40**, pp. 117-129.
- KIRK, R. & LEWIS, D. J. (1947). Studies in leishmaniasis in the Anglo-Egyptian Sudan. IX. Further observations on the sandflies (*Phlebotomus*) of the Sudan.—*Trans. R. Soc. trop. Med. Hyg.*, **40**, pp. 869-888.
- KIRK, R. & LEWIS, D. J. (1948). Taxonomy of the Ethiopian sandflies (*Phlebotomus*). III. New species and records: alterations and additions to the keys.—*Ann. trop. Med. Parasit.*, **42**, pp. 322-333.
- LEWIS, D. J. (1947). General observations on mosquitos in relation to yellow fever in the Anglo-Egyptian Sudan.—*Bull. ent. Res.*, **37**, pp. 543-566.
- LEWIS, D. J. & KIRK, R. (1946a). Five new species of *Phlebotomus* (Diptera, Psychodidae) from the Sudan.—*Proc. R. ent. Soc. Lond.*, **15**, pp. 55-60.
- LEWIS, D. J. & KIRK, R. (1946b). The male of *Phlebotomus serratus* Parrot and Malbrant (Psychodidae, Diptera).—*Ibid.*, pp. 61-62.
- LEWIS, D. J. & KIRK, R. (1949). The zoogeography of the Ethiopian species of *Phlebotomus* Agassiz (Diptera, Psychodidae).—*Proc. R. ent. Soc. Lond.* (A) **24**, pp. 51-55.
- PARROT, L. (1948a). Notes sur les phlébotomes. LVIII. Phlébotomes du Soudan Anglo-Egyptien. 1.—*Arch. Inst. Pasteur Algér.*, **26**, pp. 121-148.
- PARROT, L. (1948b). Notes sur les phlébotomes LIX. Phlébotomes du Soudan Anglo-Egyptien. 2.—*Ibid.*, pp. 259-276.
- SUDAN MEDICAL SERVICE. Annual reports for 1926, 1927, 1928 and 1932.—Khartoum.
- THEODOR, O. (1948). Classification of the Old World species of the subfamily Phlebotominae (Diptera, Psychodidae).—*Bull. ent. Res.*, **39**, pp. 85-115.
- TOTHILL, J. D. Ed. (1948). Agriculture in the Sudan.—974 pp. London, Oxford Univ. Pr.
- WAXSON, M. (1942). Sur la biologie des phlébotomes congolais.—*Rec. Trav. Sci. méd. Congo belge*, **1**, pp. 23-43.



SOME OBSERVATIONS ON THE CONTROL OF THE BONT TICK, *AMBLYOMMA HEBRAEUM* KOCH.

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(PLATES XV and XVI.)

Arsenical dips have been used in South Africa for many years to control those species of ticks that are vectors of cattle diseases. These dips have many disadvantages and, when the one-host blue tick, *Boophilus decoloratus* (Koch), developed a resistance to arsenic (Whitnall & Bradford, 1947) intensive search had to be made for other insecticides. "Gammexane", of which the active principle is the gamma isomer of hexachlorocyclohexane, has been shown to possess remarkable tick killing properties, and dips containing this insecticide have been used successfully in the field against the one-host arsenic-resistant blue tick (Thorburn, 1947; Whitnall & Bradford, 1949). Two- and three-host ticks present a different problem and the use of "Gammexane" against these has not been fully investigated. Wilson (1948) reported favourably on its use against *Rhipicephalus appendiculatus* Neum. and *Amblyomma variegatum* (F.) in East Africa. Portman (1947) has shown that in Missouri, U.S.A., the insecticide will kill all forms of the lone star tick, *Amblyomma americanum* (L.).

The control of the bont tick, *Amblyomma hebraeum* Koch, a vector of "heartwater", a disease of cattle, sheep and goats in South Africa, is a matter of great economic importance. These ticks also cause abscesses which may result in cases of myiasis, in addition to the losses caused by deaths from "heartwater". Further, sites of attachment of bont ticks are often visible on tanned hides, rendering portions of them unsuitable for use. The bont tick is a three-host tick, and its life history under natural conditions is not fully understood. Larvae and nymphs seem to occur on cattle to a lesser degree than adults, but each stage shows a definite preference for particular sites on the host (Pl. XV, fig. 1). Control measures have to be directed mainly against the adult stage.

The present paper deals with the control of the bont tick, *A. hebraeum*, with "Gammexane", arsenical dips and a combination of the two. A combination of "Gammexane" and DDT has also been investigated. Methods of applying the insecticides have been studied and comparisons made between the effects of dipping and spraying. The experiments discussed were spread over a period of two consecutive years.

Method.

Weekly counts of adult male and female bont ticks were made on treated and untreated animals, prior to each weekly treatment. It was necessary to cast the animals to enable these counts to be made, as the adults are attached mainly to the inguinal region, belly and brisket (Pl. XV, fig. 2).

The first series of experiments started in February and continued until May 1948. Twenty cattle at the farm "Paardekraal" were divided into four groups of five. Three of these groups were subjected to weekly dipping or spraying, while the fourth was left as an untreated control. The results of these treatments could be directly compared, as all twenty experimental animals grazed in the same camp, which was heavily infested with bont ticks. Ten experimental cattle were distinctively

marked on each of three other farms, "Langholm Estates" (2 dipping tanks), "Willow Fountain" and "Tharfield". Five were dipped each week and the other five left undipped as control animals. The experimental animals were allowed to graze with their respective herds. The weekly counts of ticks on dipped and undipped animals gave an indication of the effect of the treatments.

The second series of experiments began in October 1948 and continued until March 1949. Four farms, "Tharfield", "Paardekraal", "Kasouga Farm" and "Barville Park" were used in this series and, in each case, bont tick counts on treated groups of five animals were compared with counts on untreated control groups. In addition, each experiment had a group of five animals from which all males and females were removed and counted each week. These latter groups gave the rates at which cattle became re-infested with bont ticks on the respective farms, and this information was important when assessing the value of insecticidal treatments.

The dipping tanks used were of the "jump in" variety. Sprays were applied by means of an "Eclipse" double-action hand pump fitted with "Vermorel" nozzles. Twenty gallons of spray material were used for each group of five animals, and this was sufficient to wet each beast thoroughly.

In the first series of experiments, the number of males and females only were counted, but in the second series the females were further classified into unengorged and engorging individuals. The data relating to these counts for the various experiments are presented in a series of histograms (figs. 1-5).

The cattle were always dipped or sprayed at weekly intervals. Weekly records were kept of the number of animals that passed through each tank, and the periodical replenishments were also noted. Samples of dip-wash were taken for chemical analyses after each weekly dipping. These were analysed for total hydrolysable chlorine, and the gamma isomer content estimated from these determinations on the basis that the crude hexachlorocyclohexane (HCH) contained 10 per cent. of the active principle. Biological tests, using the *in vitro* technique for adult female blue ticks described by Whitnall and Bradford (1947), were also conducted with each sample of dip-wash. Samples of the various freshly mixed spray-washes were tested similarly. These data are presented in a series of tables.

Any standard insect could have been used for the *in vitro* tests, which were carried out to determine the biological activity of the washes, and as a check on chemical analyses (Whitnall & others, 1948). The technique for blue ticks was well established, and they were selected as the test arthropod as supplies were readily obtained. Bont ticks were not used because it was impossible to obtain large numbers of adult females in a uniform state of engorgement, and they were not suitable for *in vitro* work.

The term "dip" has been used to define concentrated products and, when diluted with water and used in dipping tanks, the mixtures have been referred to as "dip-washes". When freshly mixed and applied by pumps, the term "spray-wash" has been used.

First Series of Experiments—1948.

These were of a preliminary nature but informative results were obtained. They have been briefly reviewed by Whitnall & others (1949), and formed the basis of the second series. At "Paardekraal", where 20 experimental animals were used, one group of five was subjected to weekly dipping in 50 parts per million gamma isomer, the second was sprayed with the same concentration of freshly mixed "Gammexane" dip, the third was sprayed with 0.16 per cent. As_2O_3 , while the fourth was left as an

untreated control. The results of these treatments are presented in fig. 1. They were so striking that all experiments in the second series were based on the same layout.

On the other farms, where ten experimental animals were used, the treatments were as follows: Tank 2 at "Langholm" contained 100 p.p.m. gamma isomer, "Willow Fountain" had 50 p.p.m., while tank 1 at "Langholm" contained 50 p.p.m. and 0.16 per cent. As_2O_3 . The tank at "Tharfield" contained 0.16 per cent. As_2O_3 only. A "Gammexane" paste dip was used throughout the series.

Spraying and dipping with arsenic at "Paardekraal" and "Tharfield" respectively had similar effects on male and female bont ticks, and indicated that spraying and dipping with 0.16 per cent. As_2O_3 were comparable.

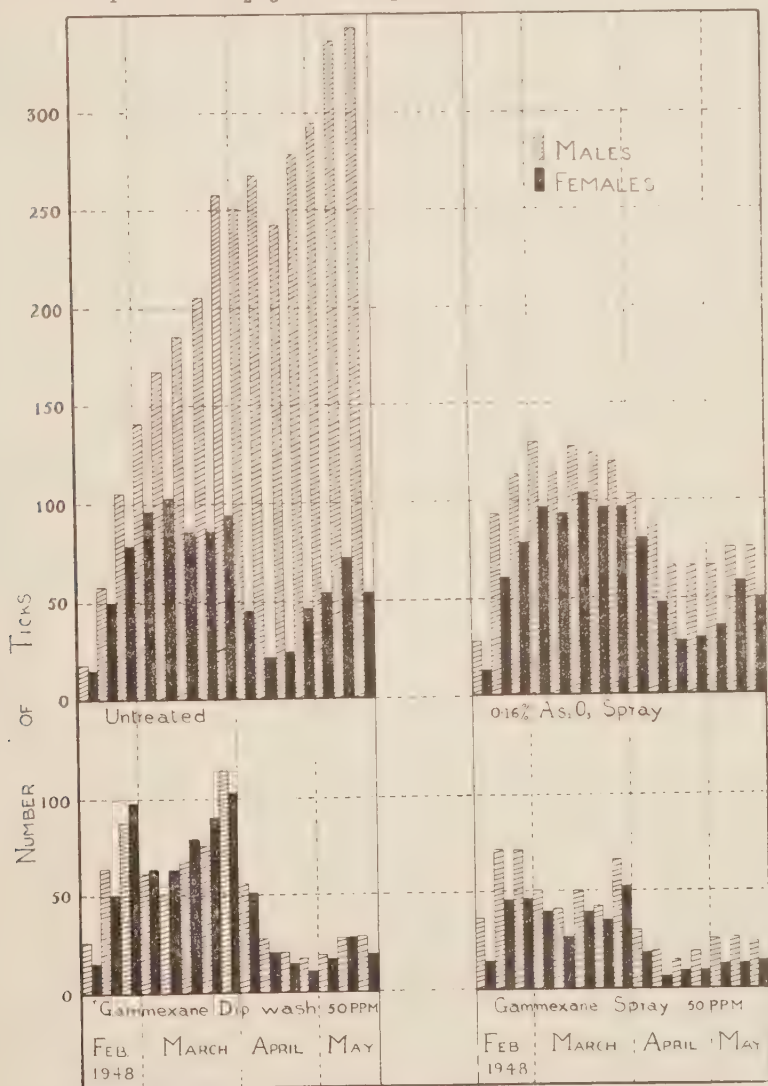


Fig. 1.—Average weekly counts of male and female bont ticks on four groups of cattle grazing in the same camp as "Paardekraal" but receiving different treatments.

Second Series of Experiments—1948-49.

In the second series of experiments, an attempt was made to find farms which could provide camps with similar infestations of bont ticks. Four farms were chosen which seemed to provide such conditions; three of them, "Paardekraal", "Tharfield" and "Barville Park", had very similar infestations, but the infestation on the fourth, "Kasouga Farm", proved to be much lighter.

"Paardekraal".

The second experiment at "Paardekraal" began on 21st October 1948 when the tank was filled with a dispersible paste dip to give an estimated concentration of 90 p.p.m. gamma isomer. Copper sulphate was added at the rate of 3 lb. per 1,000 gallons water in an unsuccessful attempt to prevent the development of putrefying matter in the wash. Some 600 head of cattle were dipped each week together with five experimental animals. Tick counts continued for 22 weeks, whilst the dipping tank remained under observation for 25 weeks. Records were taken each week from: (1) Five untreated animals, (2) five dipped in a wash containing about 100 p.p.m. gamma isomer, (3) five sprayed with a freshly mixed dilution of the paste containing about 100 p.p.m. gamma isomer, (4) five sprayed with 0.16 per cent. As_2O_3 solution, whilst (5) from five untreated animals all the adult bont ticks were removed each week. These counts are recorded in fig. 2. The males and females collected from the five animals in (5) were mounted and are shown in Pl. XVI. This affords a striking illustration of the rate at which the ticks became attached to cattle, and the density of the tick population. It will be noted that, with the exception of the first week recorded, the males always outnumbered the females. These observations did not confirm those of Lounsbury (1899) who stated, "The two sexes appear in approximately equal numbers". The collections were taken to represent the rate at which cattle became re-infested, and formed a basis on which to gauge the effectiveness of treatments. Males increased in numbers week by week on other untreated control groups of cattle, and eventually greatly outnumbered the females. This suggested that males remained on the hosts longer, and were recorded more than once in the consecutive weekly counts. This confirmed Lounsbury's observations that, "The male remains on many weeks."

Treatment with arsenic reduced the numbers of males thus confirming previous results. The numbers of females and the proportion that were engorging were very similar on untreated and arsenic-treated groups. On two occasions, 20 partly engorged females were removed from the untreated, and a similar number from the arsenic sprayed animals an hour after treatment. Seventy and one hundred per cent. of those removed from the untreated group laid eggs; in the first case all eggs hatched, but in the second only 60 per cent. Five and ten per cent. of those removed from the arsenic sprayed animals laid, but the eggs did not hatch, the control thus being 100 per cent. in both cases. These results suggest that weekly treatment of cattle with 0.16 per cent. As_2O_3 should ultimately control bont ticks on a farm. The treatment does not effect a reduction in the numbers of females attaching, but it does appear to inhibit egg laying to a marked degree, and that, even when laid, the eggs are not viable. Arnold (1949) recorded similar observations in Jamaica, where arsenious oxide solutions in strengths greater than 0.10 per cent. prevented the laying of viable eggs by *Boophilus annulatus* var. *microplus* (Can.).

On the other hand, the use of "Gammexane" either as a dip or a spray markedly reduced the numbers of both males and females, and for the first six weeks of the experiment the numbers of ticks on both treatment groups were almost the same as the numbers that were removed each week. This was satisfactory and indicated that the weekly treatments killed all ticks that attached each week, but the dip was inferior to the freshly mixed spray, from the seventh week (December) onwards. The spray was not, however, entirely satisfactory as, during January, February and

TABLE I.

Chemical and biological data relating to dip and spray samples taken from "Paardekraal".
 "Gammexane" dispersible paste containing 50 per cent. hexachlorocyclohexane, diluted 10 lb. to 500 gals. water.
 Twenty ticks treated in each sample and in water.

Sample date	Replenishments			Chemical Data			Biological Data					
	No. of cattle dipped	Water gals.	Cu. SO ₄ lb.	Dip lb.	Dip-wash gamma isomer p.p.m.	Spray gamma isomer p.p.m.	Spray As ₂ O ₃	Dip-wash		"Gammexane" Spray		Water Control
								γ γ laid	γ γ hatched	γ γ laid	γ γ hatched	
1 21/10/48	180	5,000	15	100	90	102	0.16	10	0	100	0	0
2 28/10/48	1,024	—	—	10	91	109	0.16	5	0	100	0	0
3 4/11/48	602	—	—	—	91	107	0.16	0	0	100	0	0
4 11/11/48	586	—	—	—	88	106	0.16	0	0	100	0	0
5 18/11/48	614	1,500	4½	30	81	106	0.16	10	0	100	5	5
6 25/11/48	628	100	—	—	85	106	0.16	15	0	100	0	0
7 2/12/48	580	100	—	—	79	101	0.16	5	0	100	0	0
8 9/12/48	618	—	—	—	79	95	0.16	40	25	90	5	5
9 16/12/48	601	1,300	4½	30	81	106	0.16	10	0	100	0	15
10 23/12/48	618	200	—	—	72	107	0.16	25	40	90	5	5
11 30/12/48	611	—	—	—	73	106	0.16	35	71	75	0	5
12 6/1/49	631	50	—	—	72	103	0.16	55	100	45	10	5
13 13/1/49	626	50	—	—	72	101	0.16	5	0	100	10	0
14 20/1/49	613	1,250	4½	30	72	101	0.16	5	0	100	5	5
15 27/1/49	633	—	—	—	80	90	0.16	30	50	85	5	0
16 3/2/49	616	—	—	—	76	104	0.15	55	91	50	10	5
17 10/2/49	657	50	—	—	79	101	0.16	50	100	50	0	0
18 17/2/49	627	150	—	—	77	101	0.16	55	100	45	0	0
19 24/2/49	630	1,750	6	40	87	106	0.16	5	0	100	10	5
20 3/3/49	596	—	—	—	86	114	—	25	80	20	5	5
21 10/3/49	604	—	—	—	85	104	0.16	30	100	70	10	0
22 17/3/49	667	—	—	—	85	—	—	85	72	65	—	0
23 23/3/49	415	—	—	—	81	—	—	35	86	70	—	0
24 30/3/49	514	—	—	—	85	—	—	50	90	55	—	0
25 7/4/49	280	—	—	—	88	—	—	85	100	15	—	0

March, the treated animals had more ticks each week than were removed from the control group. Engorging females on the sprayed group indicated that 100 p.p.m. gamma isomer was the minimum concentration that should be contemplated for bont tick control.

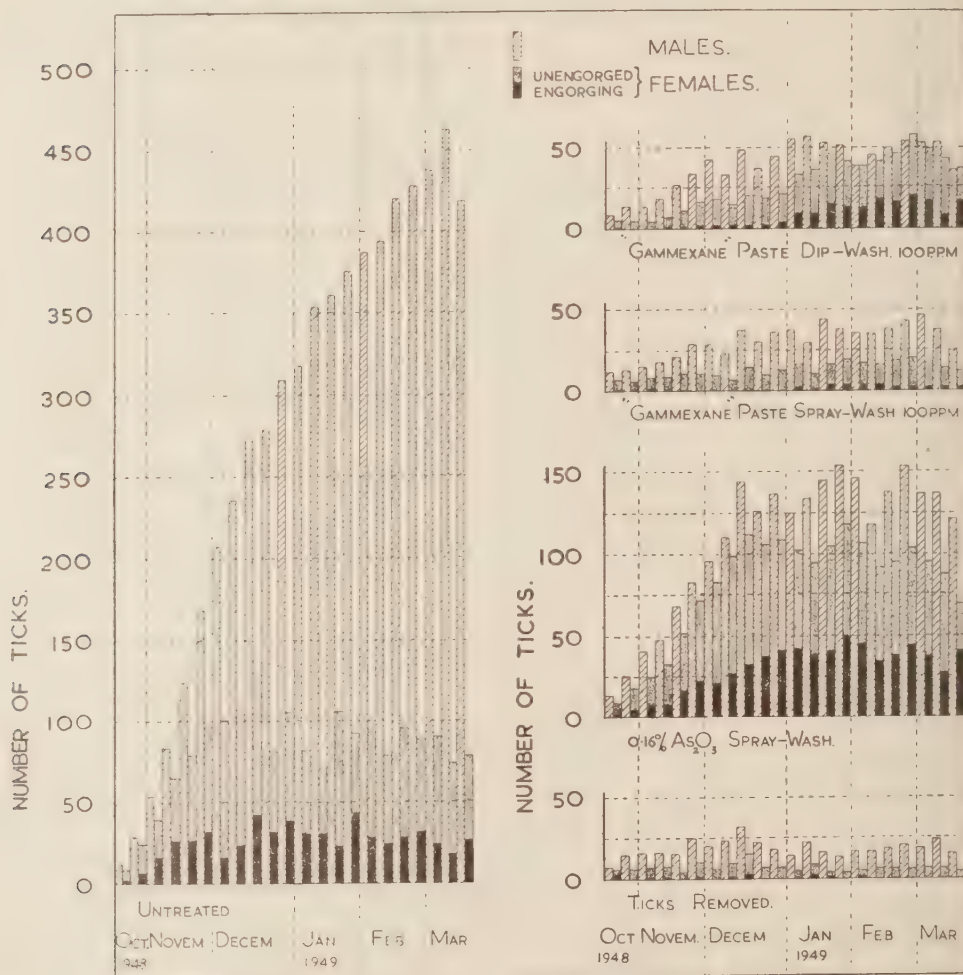


Fig. 2.—Average weekly counts of male, female and engorging female bont ticks on untreated and treated groups of cattle, compared with the average numbers removed each week from another untreated group. All cattle grazed in the same camp at "Paardekraal".

The comparative ineffectiveness of the "Gammexane" dip-wash can be better appreciated by reference to Table I. The biological tests of the samples with blue ticks ranged from 100 per cent. to 15 per cent. control, despite the fact that the estimated gamma isomer content ranged from 91 to 72 p.p.m. The loss of biological activity was first noticed towards the middle of December, and from then onwards there was a steady deterioration which was temporarily arrested by replenishments. Although freshly mixed spray-wash samples had slightly higher gamma isomer contents, the constant 100 per cent. control which these samples gave on biological

TABLE II.

Chemical and biological data relating to dip and spray samples taken from "Tharfield", "Gammexane" emulsion dip containing 25 per cent. hexachlorocyclohexane used at:—
 1: 500+1 gal. per 500 cattle dipped 19/10/48-23/11/48
 1: 250+1 gal. per 250 cattle dipped 30/11/48-29/3/49
 Twenty ticks treated in each sample and in water.

Sample date	No. of cattle dipped	Replenishments		Chemical Data		Biological Data				
		Water gals.	Dip gals./pts.	Dip-wash gamma isomer p.p.m.	Spray gamma isomer p.p.m.	Dip-wash		"Gammexane" Spray		Water
						o/o laid	o/o Batches hatched	o/o laid	o/o Batches hatched	
1a	27	4,000	8	52	57	5	0	0	0	0
1b	68	—	1 1/4	53	—	—	—	—	—	—
2	493	275	1 1/5	40	48	25	20	10	50	95
3	492	350	1	41	48	37	71	5	0	100
4	492	—	1	38	49	35	57	5	0	100
5	492	125	1 1/2	36	49	35	72	15	0	100
6	492	750	2 1/4	40	48	35	57	10	0	100
7a	30	50	10	98	95	10	50	10	0	100
7b	465	—	—	95	—	15	0	—	—	—
8	479	400	3 1/5	94	98	42	38	30	33	90
9	479	500	4	93	98	5	0	0	0	100
10	349	300	3 1/2	94	97	35	100	0	0	100
11	349	—	2	85	94	10	100	5	0	100
12	449	400	3 1/4	89	95	15	68	10	100	90
13	439	350	3 3/4	95	98	5	0	10	0	100
14	442	350	3 3/4	105	100	5	100	0	0	100
15	442	225	2 7/8	106	97	0	0	0	0	100
16	442	500	4	111	98	30	100	10	0	100
17	457	250	3	111	94	25	60	10	0	100
18	462	250	3	111	95	0	0	5	0	100
19	465	300	3 1/2	112	98	10	0	0	0	100
20	464	250	3	110	102	15	67	25	60	85
21	485	250	3	114	—	15	67	—	—	—
22	486	200	3 2/6	109	—	10	0	—	—	—
23	486	500	4	109	—	15	100	—	—	—
24	486	—	2	111	—	0	0	—	—	—

test, cannot be attributed to this factor alone. The use of the new insecticides as dips and sprays has been discussed by Whitnall & others (1948), who explained why superior results were given by the application of freshly diluted sprays as compared with dipping in dirty dip-washes, each with comparable estimated gamma isomer contents. This aspect of the problem falls outside the scope of the present paper, but the indications were, that for tick control with the new insecticides, spraying had definite advantages over dipping.

"Tharfield".

A "Gammexane" oil emulsion dip was used at "Tharfield" at the rate of 1 gallon to 500 gallons water (50 p.p.m. gamma isomer) plus 1 gallon of the same dip for every 500 head of cattle dipped, for the period 19th October to 23rd November, 1948, and at double this rate (100 p.p.m.) from 30th November, 1948 to 29th March, 1949. Previous experience with emulsions (Whitnall & Bradford, 1949) had shown that the gamma isomer content of washes containing these dips dropped with use, consequently the content was supplemented after every 500 cattle dipped to counteract this tendency. The data presented in Table II show that this was successful in maintaining the wash at the desired concentrations, and *in vitro* tests with blue ticks showed that it remained biologically active. Freshly mixed sprays gave generally better results than dirty samples from the dip tank.

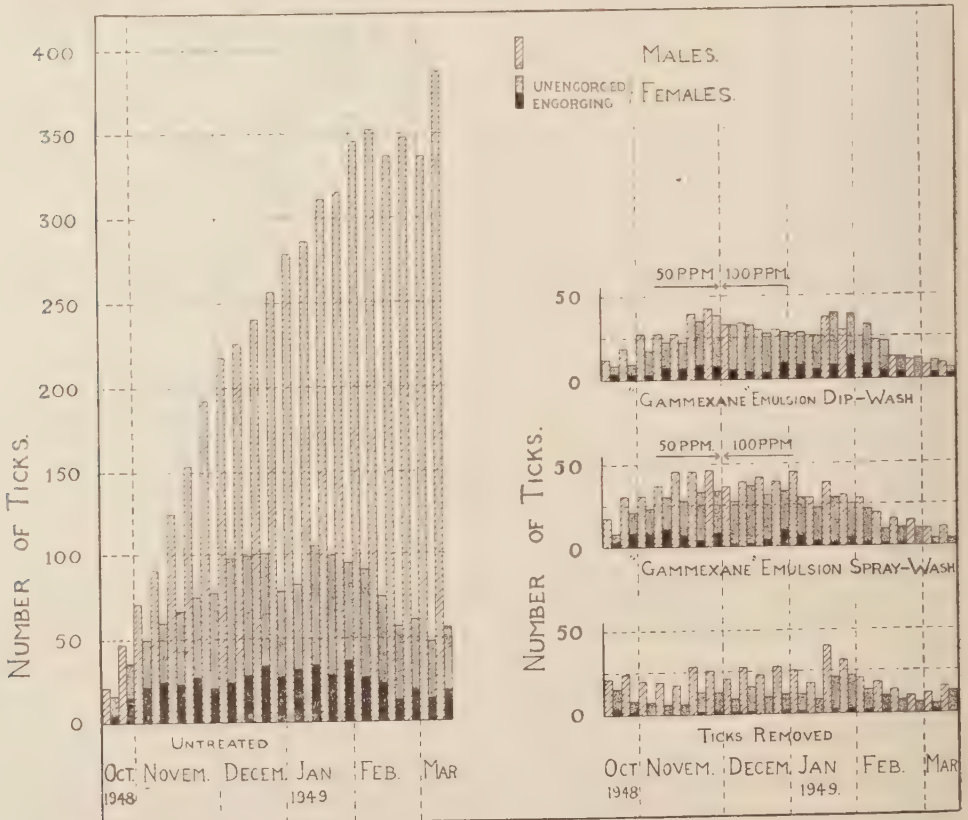


Fig. 3.—Average weekly counts of male, female and engorging female bont ticks on untreated and treated groups of cattle, compared with the average numbers removed each week from another untreated group. All cattle grazed in the same camp at "Tharfield".

TABLE III.

Chemical and biological data relating to dip and spray samples taken from "Barville Park".
 Tank filled with composite wash of 0.16 per cent. As_2O_3 plus "Gammexane" emulsion containing 25 per cent. hexachlorocyclohexane,
 1:500 and 1 gal. per 500 cattle dipped.
 Twenty ticks treated in each sample and in water.

Sample date	Replenishments			Chemical Data			Biological Data							
	No. of cattle dipped	Water gals.	Arsenical dip gals.	"Gammexane" dip gals.	Dip-wash		Spray gamma p.p.m.	Spray % As_2O_3	Dip-wash		"Gammexane" Spray		Water Control	
					isomer p.p.m. & As_2O_3	gamma p.p.m.			XX laid	Batches hatched	% Control	Batches hatched		% Control
1a 8/11/48	40	4,500	11½	9	45	0.15	48	0.15	10	100	10	0	100	0
1b 8/11/48	293	—	—	—	44	0.15	—	—	20	0	40	38	85	0
2 15/11/48	591	—	—	1	44	0.15	46	0.15	30	0	5	0	100	5
3 22/11/48	436	—	—	1	44	0.15	48	0.15	15	95	0	0	100	5
4 29/11/48	424	—	—	1	44	0.14	49	0.16	30	33	10	67	80	0
5 6/12/48	420	1,000	2½	3	42	0.14	46	0.15	65	65	30	91	50	15
6 13/12/48	426	—	—	1	43	0.14	47	0.15	65	77	55	86	70	5
7 20/12/48	433	—	—	1	40	0.13	47	0.16	75	87	35	83	75	5
8 27/12/48	443	40	1½	1	33	0.19	49	0.13	80	100	30	67	90	5
9 3/1/49	431	960	2½	3	37	0.16	45	0.14	40	33	20	100	80	5
10 10/1/49	437	—	—	1	40	0.15	48	0.15	84	75	5	0	100	0
11 17/1/49	433	195	—	1	40	0.15	48	0.15	84	37	15	100	85	0
12 24/1/49	433	1,000	2½	3	49	0.15	48	0.15	25	20	40	100	60	5
13 31 1/49	436	—	—	1	49	0.16	49	0.15	50	60	5	100	60	5
"Gammexane"-DDT Spray														
14 7/2/49	435	—	—	1	50	0.14	357	0.15	35	57	0	0	100	0
15 14/2/49	446	—	—	1	51	0.15	307	0.16	35	43	0	0	100	5
16 21/2/49	433	1,000	2½	3	47	0.14	329	0.15	25	25	0	0	100	5
17 28 2/49	437	—	—	1	47	0.14	321	0.16	85	88	0	0	100	5
18 7/3/49	442	—	—	1	49	0.16	319	0.15	35	43	0	0	100	5
19 14/3/49	452	—	—	1	50	0.16	321	0.15	10	50	5	0	100	0
20 21/3/49	237	1,000	—	3	50	0.13	—	—	40	50	—	—	—	0

Counts were recorded from untreated, dipped and sprayed animals, five in each case, whilst from a fourth group of a similar number of animals, all adult males and females were removed each week for 21 weeks. The results are presented in fig. 3.

The ticks increased in number at 50 p.p.m. gamma isomer, and formed clusters on both dipped and sprayed groups. No increase took place at 100 p.p.m. but the

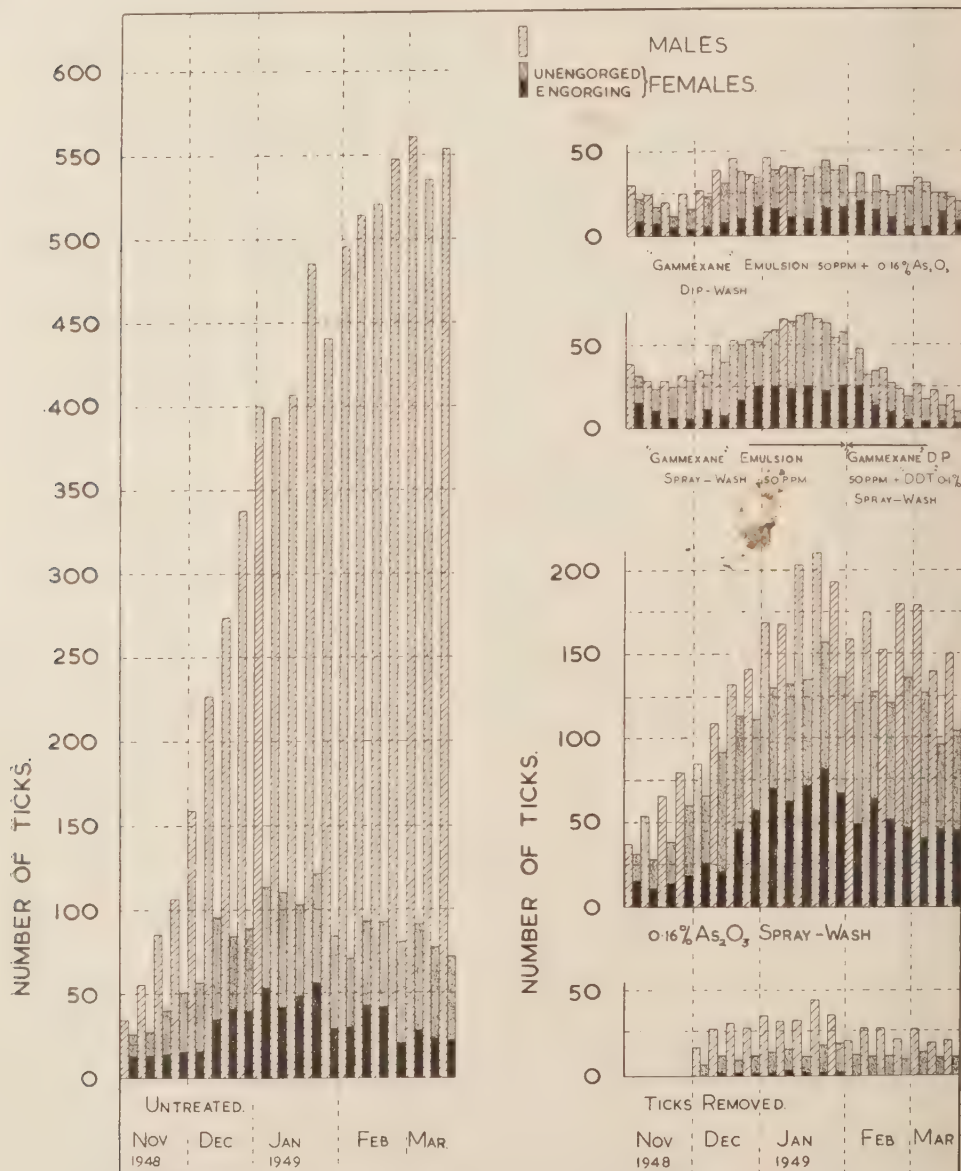


Fig. 4.—Average weekly counts of male, female and engorging female bont ticks on untreated and treated groups of cattle, compared with the average numbers removed each week from another untreated group. All cattle grazed in the same camp at "Barville Park".

clusters persisted, and were not dispersed until the middle of February 1949. The ticks were under control on both dipped and sprayed groups during the last two weeks in February and the first two in March, one month before the termination of the experiment; the numbers counted were about the same as those removed each week from the control group. The results of this experiment were considered satisfactory. If the dip had been used at 100 p.p.m. gamma isomer from the start, it is likely that clusters of ticks with engorging females would not have formed. The results obtained by dipping and spraying were very similar, and suggested that the new insecticides could be used in dipping tanks, provided frequent additions of fresh dip were made.

"Barville Park".

A composite wash consisting of 1 gallon of a "Gammexane" emulsion dip to 500 gallons water, and 1 gallon of arsenical dip to 400 gallons water, was used in the tank at "Barville Park". The gamma isomer content was maintained at 50 p.p.m. by weekly additions of 1 gallon of emulsion dip for every 500 cattle dipped. The concentration remained fairly constant as is shown by the analyses presented in Table III. In the biological tests the "Gammexane" emulsion spray samples on the whole gave better results than the samples of dip. The dispersible powder "Gammexane"-DDT spray gave excellent control.

Ticks were counted on untreated, arsenic sprayed, and "Gammexane" dipped and sprayed animals. For the last five weeks of the experiment, this latter group was sprayed with a dispersible powder preparation containing 50 p.p.m. gamma isomer and a 0.1 per cent. DDT. All the males and females were removed and counted each week from the beginning of December from a fifth group of animals. In all cases, the groups consisted of five cattle. The counts are presented in fig. 4.

The arsenic spray treatment confirmed previous findings. The results, however, were not so unfavourable as the histograms depict, for 95 per cent. of the engorging females laid after removal from the untreated group on 17.i.1949, compared with only 5 per cent. from the host animals one hour after they had been sprayed with arsenic. In the former case, all eggs hatched, but in the latter all were sterile, the control being 100 per cent.

The results of both dip (50 p.p.m. gamma isomer and 0.16 per cent. As_2O_3) and spray (50 p.p.m. gamma isomer) were not satisfactory, as at all times there were more ticks, including many engorging females, on the treated animals than were removed each week from those of the control group. The composite spray of "Gammexane" and DDT took some time to disperse the clusters of ticks which had formed during the previous treatment, but when the experiment was terminated in March, the combination treatment had brought the ticks nearly under control.

"Kasouga Farm".

The dipping tank at "Kasouga Farm" was filled with a dispersible powder dip as shown in Table IV. Counts continued for 20 weeks and were recorded from untreated and dipped animals, animals sprayed with fresh dilutions of the dispersible powder containing 100 p.p.m. gamma isomer, and others sprayed with fresh dilutions of a dispersible powder containing 50 p.p.m. gamma isomer and 0.1 per cent. p,p' DDT. From a fifth group all adult males and females were removed and counted each week. The counts are recorded in fig. 5.

TABLE IV.

Chemical and biological data relating to dip and spray samples taken from "Kasouga Farm".
 Fifty per cent. HCH dispersible powder (3 lb./100 gals.) 1/11/48-27/12/48.
 Forty per cent. HCH dispersible powder (3½ lb./100 gals.) 3/1/49-14/2/49.
 Fifty per cent. HCH dispersible powder (3 lb./100 gals.) 21/2/49-18/4/49.
 Twenty ticks treated in each sample and in water.

Sample date	No. of cattle clipped	Replenishments			Chemical Data		Biological Data			
		Water gals.	Dip lb.	Dip-wash gamma isomer p.p.m.	Spray gamma isomer p.p.m.	Spray DDT-mex-ane-Hydrolysable Cl.	Dip-wash		"Gammexane" Spray	
							laid	Batches hatched	laid	Batches hatched
1a	1/11/48	5,000	150	145	141	—	—	—	—	—
1b	1/11/48	—	—	132	102	—	0	0	—	—
2	8/11/48	—	—	111	102	—	0	0	—	—
3	15/11/48	200	6	108	103	310	10	0	100	100
4	22/11/48	—	—	102	104	315	10	0	100	100
5	29/11/48	1,200	36	100	104	315	5	0	100	100
6	6/12/48	100	3	97	104	312	25	20	95	100
7	13/12/48	—	—	95	106	303	0	0	100	100
8	20/12/48	500	15	101	102	318	0	0	100	100
9	27/12/48	—	—	91	93	304	10	50	95	100
10	3/1/49	1,600	60	102	87	293	20	50	90	100
11	10/1/49	—	—	102	89	283	0	100	100	100
12	17/1/49	500	—	101	97	309	35	86	70	100
13	24/1/49	—	18½	114	102	322	0	0	100	100
14	31/1/49	1,075	40½	118	99	317	0	0	100	100
15	7/2/49	240	3	116	96	337	0	0	100	100
16	14/2/49	—	—	114	101	325	0	0	100	100
17	21/2/49	1,600	48	124	104	328	0	0	100	100
18	28/2/49	—	—	126	100	325	15	67	90	100
19	7/3/49	—	—	122	98	324	5	100	95	100
20	14/3/49	—	—	119	98	318	5	0	100	100
21	21/3/49	—	—	121	—	—	25	80	80	100
22	28/3/49	—	96	140	—	—	0	0	100	100
23	4/4/49	—	—	141	—	—	5	0	100	100

The dip and the "Gammexane" spray were both used at about 100 p.p.m. gamma isomer, but less powder was necessary to give this concentration in the freshly mixed spray. In spite of this, the spray gave consistently good results in the biological tests, while the dip showed fluctuations. The fresh spray also gave better control of bont ticks than the dip, which allowed many females to become partly engorged. The "Gammexane"-DDT spray gave similar *in vitro* results to the 100 p.p.m. gamma isomer spray, but the combination treatment reduced the weekly counts of bont ticks below the number removed each week from the control group. The good results of the "Gammexane"-DDT spray suggest a synergism, a possibility also mentioned by Arnold (1949) in his work with *B. annulatus* var. *microplus*, in both laboratory experiment and field trials. They may, however, be due to persistent action or repellent effect. The comparatively low infestation on "Kasouga Farm" made it difficult to assess the full value of the treatments.

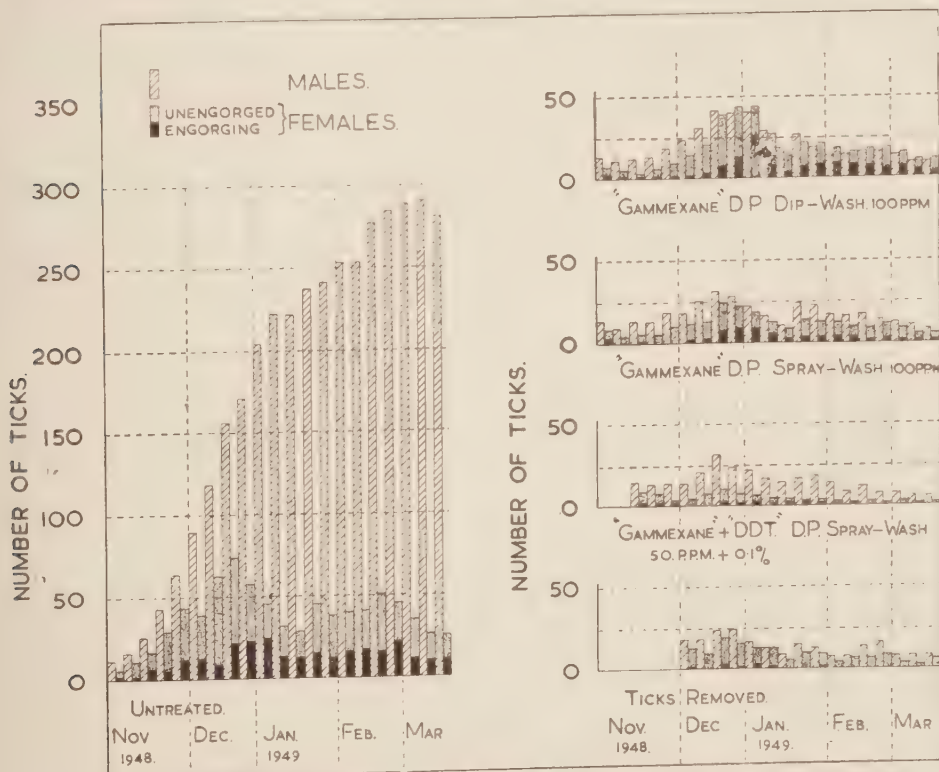


Fig. 5.—Average weekly counts of male, female and engorging female bont ticks on untreated and treated groups of cattle, compared with the average numbers removed each week from another untreated group. All cattle grazed in the same camp at "Kasouga Farm".

Summary.

"Gammexane" and DDT, have many advantages over arsenic as tick killing agents. "Gammexane" dips have been used successfully to control the one host arsenic-resistant blue tick, *B. decoloratus* (Koch) but these dips have not been fully investigated for the control of two- and three-host ticks. The control of the three-host bont tick, *A. hebraeum*, a vector of "heartwater", a disease of cattle, sheep and goats, is of great economic importance to South Africa. Larvae and nymphs seem to occur

on cattle to a lesser degree than adults, but each stage shows a definite preference for particular sites on the host. For this reason, control measures have to be mainly directed against the adult stage. The effect of "Gammexane", DDT and arsenical dips, and combinations of these, has been investigated, by making weekly counts of adults on treated and untreated groups of animals. Dipping has been compared with spraying, and the results have been examined in the light of chemical analyses and biological tests with the same samples. The experiments were spread over two consecutive years.

Preliminary experiments indicated that all treatments markedly reduced the numbers of male bont ticks on the cattle. Weekly arsenical treatments with 0.16 per cent. As_2O_3 either by dipping or spraying did not reduce the numbers of females, nor did a composite dip-wash of 0.16 per cent. As_2O_3 and 50 p.p.m. gamma isomer. Dipping in 50 p.p.m. gamma isomer gave slightly better results against females than the above treatments. Encouraging results were obtained by spraying cattle with freshly diluted wash containing 50 p.p.m. gamma isomer, but dipping in 100 p.p.m. also gave satisfactory results. The relative ineffectiveness of dipping as compared with spraying, was found to be due to the loss of biological activity of hexachlorocyclohexane in dipping tanks, where complicating pollution factors appeared.

The results of the preliminary experiments were largely confirmed by the second series. Males always outnumbered females in collections where the ticks were removed week by week from cattle. The collections were taken to represent the rate at which cattle became re-infested, and formed a basis on which to gauge the effectiveness of treatments. Males increased in numbers week by week on other untreated control groups of cattle, and eventually greatly outnumbered the females. This suggested that males remained on the hosts longer, and were recorded more than once in the consecutive weekly counts.

All treatments reduced the numbers of males. Weekly treatments in 0.16 per cent. As_2O_3 did not reduce the numbers of females, nor did it prevent them from engorging. Some females laid after removal from cattle so treated but the eggs were sterile, whilst females in a similar state of engorgement, removed from untreated animals, laid fertile eggs. Arsenical treatments should thus eventually control bont ticks.

All "Gammexane" treatments appeared more effective than the arsenical treatments. Fresh dilutions of dispersible pastes and powders in the form of sprays gave better results than dipping in the same preparations at comparable concentrations. This was due to a loss in biological activity of the hexachlorocyclohexane as the washes became dirty with use in dipping tanks. This factor makes chemical determinations of dip-washes, based on total hydrolysable chlorine, of little value, unless these data are correlated with some suitable biological test. The addition of 0.03 per cent. copper sulphate in the wash did not prevent the loss of biological activity.

Oil emulsion dips, which were known to show a drop in the gamma isomer content with use in dipping tanks, were kept at the desired concentration and biologically active by adding fresh dip each week. In such cases both dip- and spray-washes gave satisfactory results when used at 100 p.p.m. gamma isomer. A combination of 50 p.p.m. gamma isomer and 0.16 per cent. As_2O_3 used as a dip-wash was not satisfactory in reducing the number of bont ticks, and little better than a fresh spray of 50 p.p.m. gamma isomer alone. The striking results given by a combination of a dispersible powder spray of 50 p.p.m. gamma isomer and 0.1 per cent. p.p' DDT might be due to persistent action or repellent effect.

Arsenic is a stable substance and has been used for many years in dipping tanks to control ticks. It has disadvantages and in the case of the bont tick many females remained attached to the hosts when treated weekly in arsenic, although the engorged females laid sterile eggs. "Gammexane" preparations when used at 100 p.p.m.

gave satisfactory results. These preparations, however, lost their biological activity in dipping tanks, and the best results were obtained when they were applied to cattle as fresh sprays.

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References.

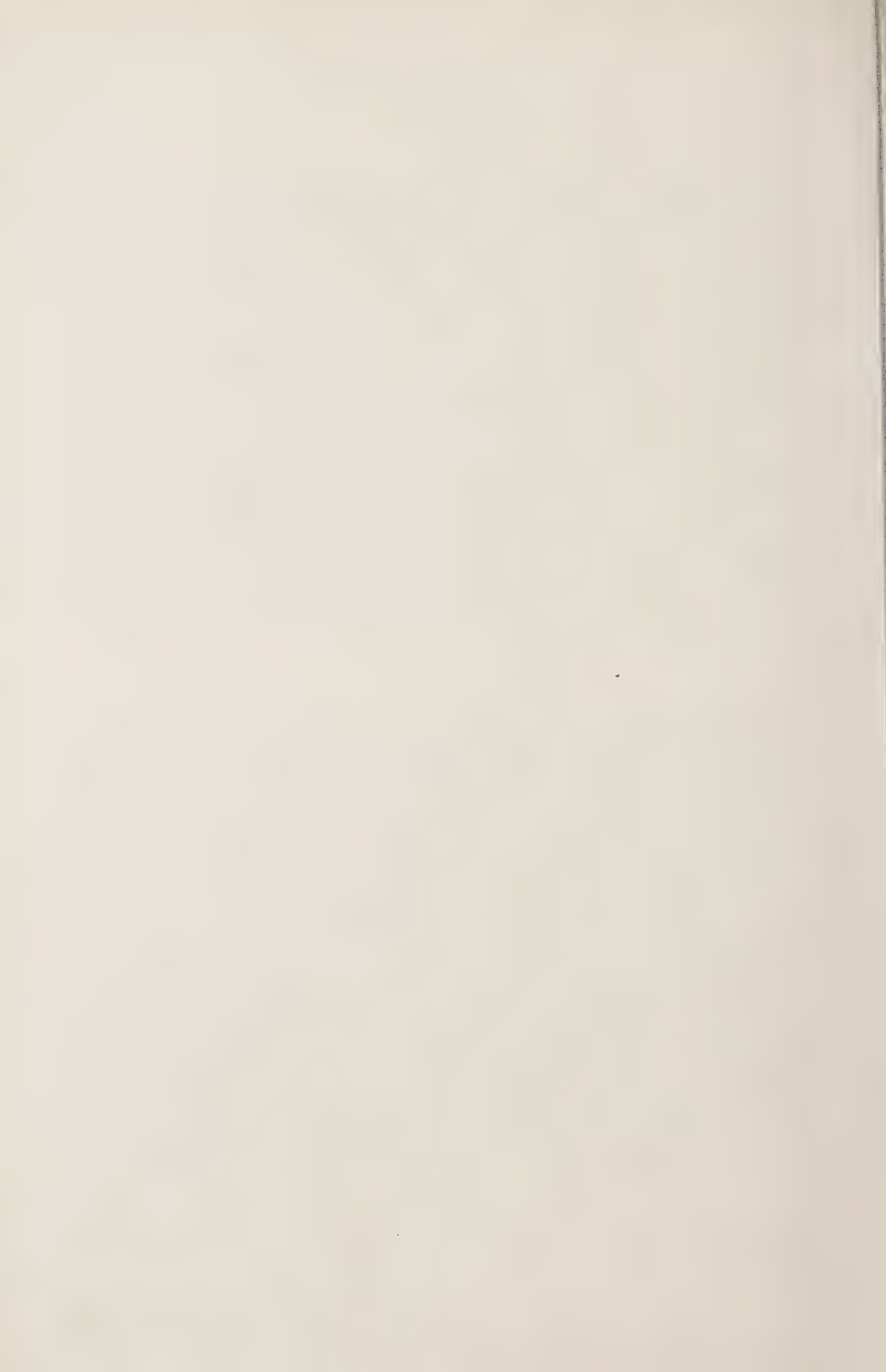
- ARNOLD, R. M. (1949). Tick control measures. Assessment of the value of chemical tickicides for *Boophilus (Margaropus) annulatus* var. *microplus* in Jamaica.—Vet. Rec., **61**, pp. 198–201, 212–217.
- LOUNSBURY, C. P. (1899). The Bont Tick, *Amblyomma hebraeum* Koch. Its life history and habits.—Agric. J. Cape of Good Hope, **15**, pp. 728–743.
- PORTMAN, R. W. (1947). Dipping in benzene hexachloride to control *Amblyomma americanum*.—J. econ. Ent., **40**, pp. 134–135.
- THORBURN, J. A. (1947). The control of ectoparasite infestations of farm stock with "Gammexane", with special reference to the arsenic-resistant Blue Tick.—Empire J. of exp. Agric., **15**, pp. 42–50.
- WHITNALL, A. B. M. & BRADFORD, B. (1947). An arsenic-resistant tick and its control with "gammexane" dips.—Bull. ent. Res., **38**, pp. 353–372.
- WHITNALL, A. B. M. & BRADFORD, B. (1949). An arsenic-resistant tick and its control with "Gammexane" dips. Part II.—Bull. ent. Res., **40**, pp. 207–226.
- WHITNALL, A. B. M., BRADFORD, B., MCHARDY, W., WHITEHEAD, G. B. & MEERHOLZ, F. (1948). Some observations on biological and chemical tests to determine the effectiveness of benzene hexachloride dip-washes.—S. Afr. Sci., **2**, pp. 112–113.
- WHITNALL, A. B. M., BRADFORD, B., MCHARDY, W., WHITEHEAD, G. B. & MEERHOLZ, F. (1949). Some preliminary observations on the control of the Bont Tick, *Amblyomma hebraeum* Koch.—S. Afr. J. Sci., **45**, pp. 115–116.
- WILSON, S. G. (1948). A method of assessing the acaricidal properties of DDT and "Gammexane" preparations in field trials.—Bull. ent. Res., **39**, pp. 269–276.

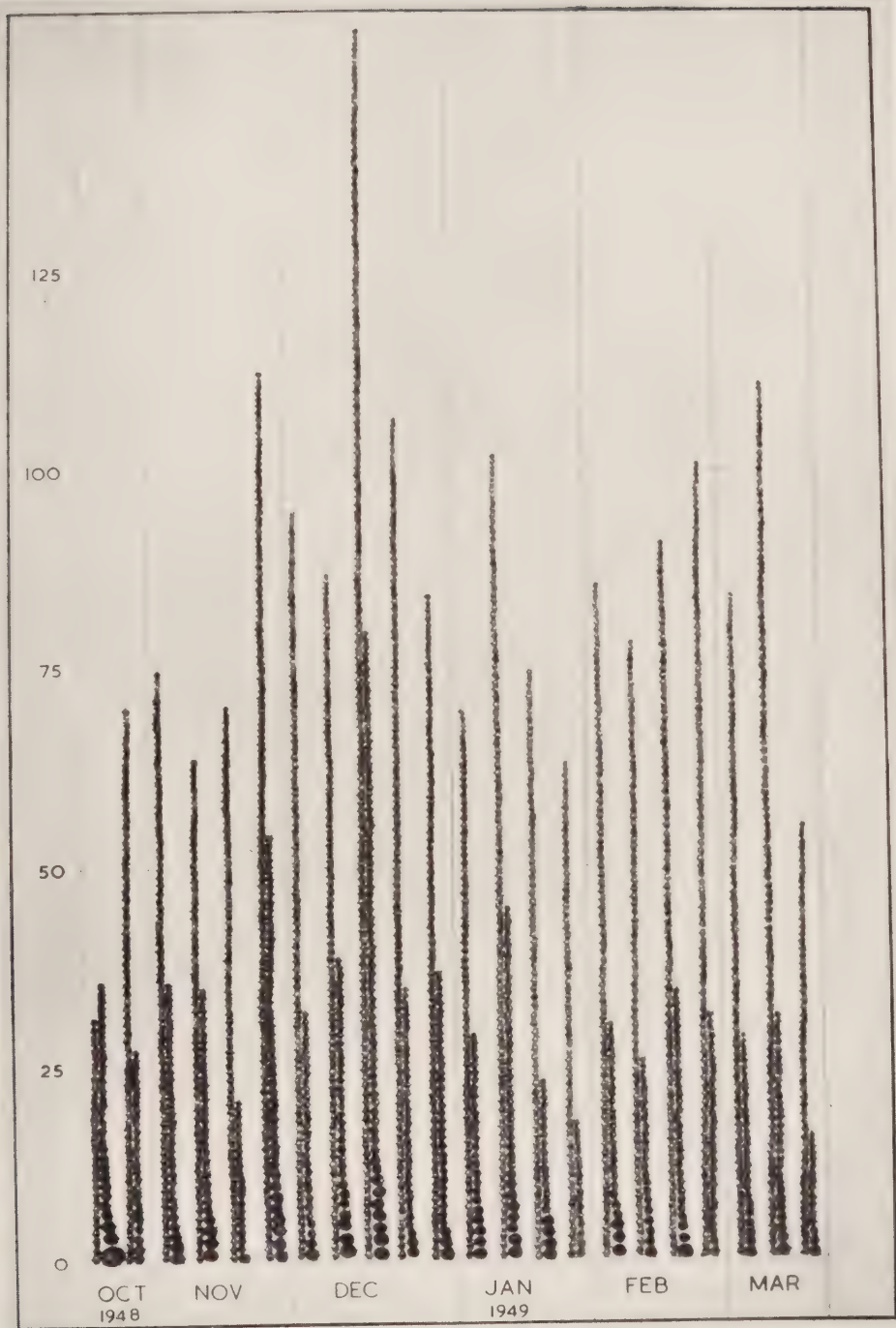


FIG. 1. Male and female bont ticks on the inguinal region of an untreated ox.



FIG. 2. Male and female bont ticks were accurately counted while animals were lying on the ground.





Showing the numbers of male and female bont ticks that attached week by week to five animals over a period of 22 weeks at "Paardekraal." Males on the left and females on the right of each column.



ON THE NARCISSUS FLIES, *MERODON EQUESTRIS* (F.) AND *EUMERUS TUBERCULATUS* (ROND.) AND THEIR CONTROL.

By E. E. EDWARDS, D.Sc., *Department of Zoology, University College of the Gold Coast*, and W. J. BEVAN, M.Sc., *late of the Agricultural Zoology Department, University College, Cardiff*.

The Narcissus flies, *Merodon equestris* (F.) and *Eumerus tuberculatus* Rond., are prevalent in most localities in Britain where Narcissus bulbs are extensively grown. The Large Narcissus Fly, *M. equestris*, was first recorded in Britain by Verrall in 1869 (Verrall, 1901) in a garden growing bulbs which had been imported from Holland. By 1896 it was a common pest in many areas and Fryer (1914a) recorded its presence in England, Wales, Scotland and Ireland. This author also indicated that it was supposed to have been introduced into Britain from Holland, but Theobald (Hodson, 1932), on the other hand, believed that the species was indigenous to Britain as it had been found by him parasitic on the Wild Hyacinth, *Scilla nutans*.

The Small Bulb Fly, *E. tuberculatus*, is probably indigenous to Southern Europe, having been recorded in Italy as early as 1857 by Rondani. This species was not discovered in Britain until 1920 when Collin gave a description of it. In the United States of America and Canada, it has also been known since 1920 (Mackie, 1922).

Serious attempts to discover means of controlling the Narcissus flies have been made in Britain. Fryer (1914a) was one of the earliest workers in this field and recommended the immersion of dormant bulbs in warm water for 24 to 48 hours. He also indicated that netting the flies could be recommended in the case of small areas of bulbs grown in gardens. Hodson (1932) developed the warm water treatment and discovered that steeping the dormant bulbs in water at 110°F. for three hours destroys, besides the Bulb and Stem Eelworm, *Anguillulina dipsaci*, the larvae of Narcissus flies, but it cannot be regarded as a satisfactory method of control as the treatment, when carried out at intervals of two or three years, does not prevent heavy re-infestations.

Various fumigants have been tested with a view to destroying the larvae within the dormant bulbs. According to Mackie (1922), the larvae of *Eumerus* spp. can be killed by fumigating the infested bulbs with carbon bisulphide for one hour, using 2 lb. per 100 cu. ft. Exposures of up to one hour had no harmful effects on the bulbs but treatment for longer periods caused damage. Hodson (1932) has shown that fumigation with calcium cyanide can also destroy the larvae of the Narcissus flies without causing injury to the bulbs. Fumigation with paradichlorobenzene is known to effect complete control of these larvae without damaging the bulbs, but, in common with carbon bisulphide and calcium cyanide, it requires special containers.

Cultural operations, such as light surface cultivation between the plants during the oviposition period, have resulted in a certain amount of success in reducing infestations. The removal of affected bulbs in the growing crop and their subsequent destruction is practised by many growers but anything up to 50 per cent. of the population often escapes death on account of the difficulty of detecting lightly affected bulbs and the fact that a high proportion of the larvae have already left the bulbs selected for destruction. The applications of strong smelling substances, such as naphthalene, between the rows of bulbs, in order to deter the flies from depositing their eggs, often results in the reduction of infestations but the chemicals hitherto tested have proved too transitory in their action to give adequate control. A poison bait, applied in the form of a spray, to destroy the adult flies has been advised by Hodson (1931 and 1932)

as a method worthy of adoption by growers. This bait consists of 4 oz. sodium arsenate, 1 lb. glycerine, 2 lb. sugar in 4 gals. of water and is sprayed over the foliage at weekly intervals during the oviposition period.

Despite all these various recommendations made from time to time, the control of Narcissus flies still remains a major problem in the British Isles. In view of these circumstances, experiments on the control of both species, *M. equestris* and *E. tuberculatus*, were carried out by the writers during 1946 and 1947 at Llandaff, Cardiff, where narcissi had been grown for many years for hybridising purposes and where each season the larvae of these flies had largely nullified all efforts in this direction, especially in the case of certain valuable hybrids. Three substances were included in these experiments, namely, dichloro-diphenyl trichlorethane (DDT), benzene hexachloride (BHC) and mercurous dichloride (Calomel).

Laboratory Experiments.

Laboratory experiments had already been conducted in 1945 when the following preparations of DDT and BHC were tested against the adult flies of both *M. equestris* and *E. tuberculatus* :—

1. DDT in the form of a 5 per cent. dust.
2. DDT in the form of a 0·5 per cent. water suspension.
3. BHC in the form of a 0·2 per cent. water suspension.

For these tests, large petri dishes lined at the bottom with filter paper and covered over at the top with fine muslin were used. Each preparation was applied in such a way that it formed a very light deposit over the surface of the filter paper. A collection of adult flies were introduced into each dish and allowed to remain for a period of one minute. The flies were then removed into glass containers free of any insecticide and kept under observation for any unusual reaction. Several batches of flies of each species were subjected in this manner to the influence of each of these three insecticidal preparations while other batches were kept as untreated controls. The results are summarised in Table I.

TABLE I.
Effect of DDT and BHC on Adults of *M. equestris* and *E. tuberculatus*.

Series	Treatment	Effect of Treatment
1	DDT, 5 per cent. dust 	Signs of paralysis in 30–60 mins. : death within 10 hours.
2	DDT, 0·5 per cent. water suspension	Signs of paralysis in 30–60 mins. : death within 10 hours.
3	BHC, 0·2 per cent. water suspension	Signs of paralysis in 25–45 mins. : death within 5 hours.
4	Control (no insecticide) 	Still alive after 24 hours confinement.

It is evident from the results shown in Table I that the adults of both species of Narcissus flies soon die on coming in contact with either DDT or BHC at the concentrations tested in these experiments, even at a strength as low as 0·2 per cent. in the case of the latter insecticide.

The effects of the same preparations of DDT and BHC were also tested under identical conditions on the larvae of these two species. In addition, a 0·5 per cent. water suspension of BHC was included. A collection of larvae was introduced into each petri dish and allowed to crawl for a duration of one minute over filter paper impregnated with the insecticide. Several batches of larvae of both species were subjected to the effects of each treatment.

No adverse effects were produced on the larvae of either species which were allowed to crawl for a period of one minute over a medium impregnated with the insecticides. All the larvae under each treatment remained active and normal in behaviour. Most of them ultimately turned into pupae from which adult flies emerged in due course. In one series of tests the larvae were allowed to remain for a period of 20 hours in contact with filter paper impregnated with 0.5 per cent. water suspension of BHC. Even after this duration of exposure, no lethal effect was produced.

In other series of experiments, batches of larvae of both species were placed in large petri dishes and sprayed with the water suspensions of DDT and BHC. The larvae were then transferred into clean glass dishes and kept under observation for several days.

Water suspensions of DDT at concentrations of 0.1 per cent. and 0.5 per cent. did not prove lethal to larvae of either *M. equestris* or *E. tuberculatus*, even when applied in such a manner that their entire bodies became covered with these sprays. Water suspensions of BHC at 0.5 per cent. and 0.2 per cent. when applied in this way, on the other hand, had a deleterious effect on the larvae, some 25 per cent. of them, on an average, being dead within about 24 hours of having been sprayed. It is considered, however, that further experiments are essential as the number of tests carried out do not justify definite conclusions.

Field Experiments.

The area selected for experimental purposes in 1946 and 1947 was about 2,000 sq. ft. and divided into 12 plots, each consisting of five rows of bulbs, 22 in. long and 18 ins. apart. The insecticides which had already proved promising in preliminary trials were included in these experiments. In both seasons, each insecticide was tested on triplicated plots arranged at random while three plots were left untreated to serve as controls. The treatments were as follows :—

Plots 1a, 1b, 1c.—Control plots, no treatment.

Plots 2a, 2b, 2c.—DDT dispersible powder applied as a spray at a concentration of 0.5 per cent. suspension in water.

Plots 3a, 3b, 3c.—BHC dispersible powder applied as a spray at a concentration of 0.5 per cent. suspension in water.

Plots 4a, 4b, 4c.—Calomel powder applied as a 4 per cent. mercurous chloride dust.

The DDT and BHC preparations were applied by means of a hand-sprayer fitted with a fine nozzle, delivering one gallon of the diluted solution per 16 yds. run of row. The calomel dust was applied with a hand-bellows at the rate of 1 lb. per 40 yds. run of row. The applications were made on the following dates, some two or three days before the normal appearance of the Narcissus flies in the district :—

DDT and BHC.—In 1946 on May 10, June 7 and July 13.

In 1947 on May 11, June 7 and July 13.

Calomel.—In 1946 on May 10, June 7 and 26, July 13 and 24.

In 1947 on May 11, June 7 and 26, July 13 and 21.

The years 1946 and 1947 were characterised by high rainfalls during May, June and July, heavy showers often occurring shortly after the application of the insecticides. Despite exceedingly heavy rains, the deposits of DDT and BHC on the foliage were found to retain their lethal effects up to, at least, four weeks after spraying. Leaves, picked at intervals up to some four weeks, from plots sprayed with these insecticides and placed in cages with Narcissus flies and Carabid beetles, proved capable of causing paralysis within 24 hours of contact and eventually death. The effectiveness of the

BHC preparation in this respect was found slightly less persistent under the conditions of these particular experiments. Specimens of numerous species of Coleoptera and Diptera were often seen on the plots sprayed with DDT and BHC (Plots 2a, 2b, 2c, 3a, 3b and 3c) either dead or suffering from paralysis. It was also frequently noticed that newly emerged adults of *M. equestris* and *E. tuberculatus*, from bulbs that had been infested when planted, remained stationary on the foliage for some 30 minutes while their wings became fully expanded. Undoubtedly, this operation did account for a high mortality of Narcissus flies on emerging from the DDT and BHC sprayed plots as it gave an opportunity for the insecticides to produce their lethal effects. The adults of both species were first observed on the wing in 1946 on May 11, and in 1947 on May 12. In neither seasons were adults of *M. equestris* seen after the middle of July but those of *E. tuberculatus* remained abundant into August.

In order to ascertain the value of the three chemicals included in these experiments for the control of infestations by Narcissus flies, the bulbs were lifted in both seasons towards the end of August when all the foliage had completely died down. The bulbs were carefully examined for the presence of the larvae of either species and the results are summarised in Tables II and III.

TABLE II.

Percentage of Narcissus Bulbs attacked by the Larvae of *M. equestris* in 1946 and 1947.

Year	Plot	Treatment			
		Control	DDT	BHC	Calomel
1946	a	26.44	—	—	1.52
	b	23.00	3.10	8.31	7.21
	c	29.01	10.00	—	2.23
	Average	26.15	4.37	2.77	3.65
1947	a	26.79	8.92	—	13.98
	b	32.03	6.47	2.12	18.41
	c	28.86	7.75	1.86	14.15
	Average	29.23	7.71	1.33	15.51
Average 2 years		27.69	6.04	2.05	9.58

The figures for the average numbers of bulbs attacked on the treated plots, shown in Table II, columns 4, 5 and 6, indicate that DDT, BHC and calomel were successful in effecting a marked reduction in the degree of infestation by the Large Narcissus Fly, *M. equestris*, both in 1946 and 1947. BHC and DDT proved the most effective, the proportion of Narcissus bulbs infested, on an average basis on the three plots for two years, being 2.05 per cent. and 6.04 per cent., respectively, compared with 27.69 per cent. for the untreated (control) plots. No definite conclusions can be reached from an examination of the results obtained in these experiments as to the relative value of BHC and DDT for the control of the Large Narcissus Fly. The figures strongly suggest, however, that the former is superior to the latter when applied in the form of a spray, at a concentration of 0.5 per cent. suspension in water, over the aerial parts of Narcissus bulbs and the surrounding soil on three separate occasions at approximately monthly intervals during the oviposition period, starting about May 10 (or two or three days before the normal appearance of the adult fly in the district). Calomel dust, on the other hand, whilst producing an appreciable reduction in the extent of infestation by the Large Narcissus Fly when applied as a 4 per cent. mercurous bichloride dust, did not exercise such a pronounced degree of control in these experiments as BHC and DDT, the percentage of infested Narcissus bulbs on

the plots so treated, on an average for the two seasons, being 9.58, compared with 27.69 on the untreated plots. The degree of protection afforded by calomel dust against attacks by this species in 1947 was considerably lower than that given by this substance in the preceding season, the percentage of affected bulbs for the three plots being 15.51 and 3.65, respectively. This marked variability in the results obtained with calomel dust in these two seasons fully confirms the experience gained by the writers in its usage for the control of Narcissus flies in previous years at this centre and in other localities in South Wales and Monmouthshire. It would seem that the inconsistency in the efficacy of calomel dust for the control of Narcissus flies is largely associated with climatic conditions at the time of its application and throughout the period when inhibition of hatching of the eggs of these flies due to its presence is expected.

TABLE III.

Percentage of Narcissus Bulbs attacked by Larvae of *E. tuberculatus*, in 1946 and 1947.

Year	Plot	Treatment			
		Control	DDT	BHC	Calomel
1946	a	11.47	—	—	7.36
	b	5.24	1.52	—	3.54
	c	6.43	—	2.15	8.92
	Average	7.71	0.51	0.72	6.61
1947	a	2.82	—	—	3.69
	b	2.76	—	—	—
	c	4.25	—	—	2.10
	Average	3.28	—	—	1.93
Average 2 years		5.49	0.25	0.36	4.27

It is evident from the figures presented in Table III that the most successful results against the Small Narcissus Fly, *E. tuberculatus*, followed the application of BHC and DDT, the percentage of infested bulbs on the plots treated with these insecticides, on an average for 1946 and 1947, being 0.36 and 0.25, respectively, compared with 5.49 on the untreated controls. Further outstanding features of the data shown in Table III are the negligible control of the Small Narcissus Fly given by calomel dust in 1946 and 1947 and the low incidence of this species in both seasons, judging by the percentage of bulbs attacked by the larvae.

The figures for the average numbers of infested bulbs on the untreated plots (column 3) in Tables II and III confirm the results of extensive field observations made by the writers that the Large Narcissus Fly, *M. equestris*, was far more abundant and destructive to Narcissus bulbs than the Small Narcissus Fly, *E. tuberculatus*, in South Wales and Monmouthshire in 1944-47. The figures for the average numbers of infested bulbs on the treated plots in these two Tables show that BHC and DDT resulted in a marked reduction in the extent of infestations by both species of Narcissus flies and that the former chemical proved the more valuable insecticide in this respect in these particular experiments. The percentages of infested bulbs irrespective of species of Narcissus fly, on an average basis for the triplicate plots under each treatment for the two years, were 2.41, 6.29 and 13.85 for the BHC, DDT and calomel treated plots, respectively, and 33.68 for the untreated controls.

Summary.

A résumé is given of the recommendations hitherto made for the control of the Narcissus flies, *Merodon equestris* and *Eumerus tuberculatus*. Nevertheless, the control

7
five
of these pests still remains a major problem in the British Isles. Laboratory and field experiments on the control of both these species were carried out in 1946 and 1947. In the field trials, dichloro-diphenyl trichlorethane (DDT), benzene hexachloride (BHC) and mercurous bichloride (calomel) were included. The DDT and BHC were applied in the form of sprays, at a concentration of 0.5 per cent. suspension in water, over the aerial parts of the Narcissus plants and the surrounding soil on three separate occasions at approximately monthly intervals during the oviposition period, starting on May 10, some two or three days before the adult flies normally appear in the district. The calomel was applied in the form of a 4 per cent. dust on six separate occasions during this period.

BHC and DDT were successful in producing a marked reduction in the degree of infestations by both species of Narcissus flies and, of the two insecticides, the former was apparently more effective. Although calomel dust afforded an appreciable protection against attacks by the Large Narcissus Fly, *M. equestris*, it exercised negligible control of the Small Narcissus Fly, *E. tuberculatus*, in these experiments. It seems from the results obtained in the present investigations that DDT and, in particular, BHC do provide a much more effective and practical means of controlling infestations of the Narcissus flies than any other methods hitherto tested.

Acknowledgements.

Grateful acknowledgements are due to Major E. David, Llandaff, Cardiff, for the facilities to conduct the field investigations upon his land and also to Mr. J. C. Mather, B.Sc., for the very valuable assistance rendered by him in connection with the 1946 experiments.

References.

- COLLIN, J. E. (1920). *Eumerus strigatus* and *tuberculatus* Rondani (Diptera, Syrphidae).—Ent. mon. Mag., **56**, pp. 102–106.
- [FRYER, J. C. F.] (1914*a*). Narcissus flies.—J. Bd Agric., **21**, pp. 136–141.
- FRYER, J. C. F. (1914*b*). Further Notes on Narcissus Flies.—*Ibid.*, **21**, pp. 424–426.
- HODSON, W. E. H. (1927). The bionomics of the Lesser Bulb Flies, *Eumerus strigatus*, Flynn., and *Eumerus tuberculatus*, Rond., in south-west England.—Bull. ent. Res., **17**, pp. 373–384.
- HODSON, W. E. H. (1931). A new method of preventing attacks of Bulb Flies on narcissus.—J. Minist. Agric., **38**, pp. 54–60.
- HODSON, W. E. H. (1932). The Large Narcissus Fly, *Merodon equestris*, Fab. (Syrphidae).—Bull. ent. Res., **23**, pp. 429–448.
- MACKIE, D. B. (1922). Note on the Lesser Bulb or Lunate Fly (*Eumerus strigatus* Fallén).—Mon. Bull. Calif. Dep. Agric., **11**, p. 759.
- RONDANI, C. (1857). Dipterologiae italicae prodromus, **2**, pp. 93–94. Parma.
- VERRALL, G. H. (1901). British flies. Vol. 8. Syrphidae, pp. 556–560. London.

A NEW INJURIOUS INDIAN GRASSHOPPER (ORTHOPTERA, ACRIDIDAE).

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The new genus *Indomerus*, described below, belongs to the group Calliptamini and is closely related to the group of genera characterised by a single tooth under the apical lobe of the cercus, but differs from all of them, except *Paracaloptenus* I. Bol. and *Peripolus* Mart., by the lobiform elytra. It differs from both *Paracaloptenus* and *Peripolus* by the broad, spade-shaped prosternal tubercle. From *Peripolus* the new genus differs also by the shape of the apical tooth of cercus, which in *Indomerus* is recurved backwards and covered by the upper lobe, while in *Peripolus* this tooth is lobiform like the upper lobe; from *Paracaloptenus* it differs by the ex-curved posterior margin of the pronotum which in *Paracaloptenus* is slightly incurved.

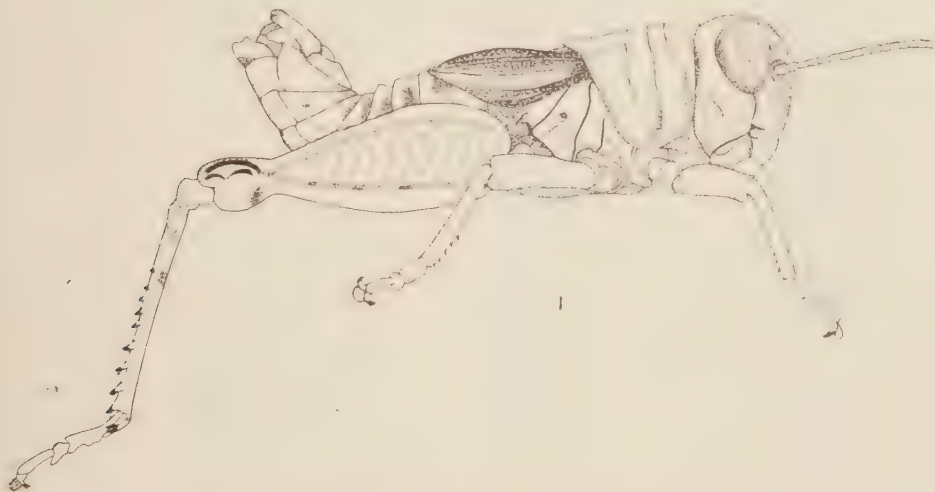


Fig. 1.—*Indomerus noxius*, gen. et sp. n. Male ($\times 4$).

Indomerus, gen. nov.

Of medium size, robust.

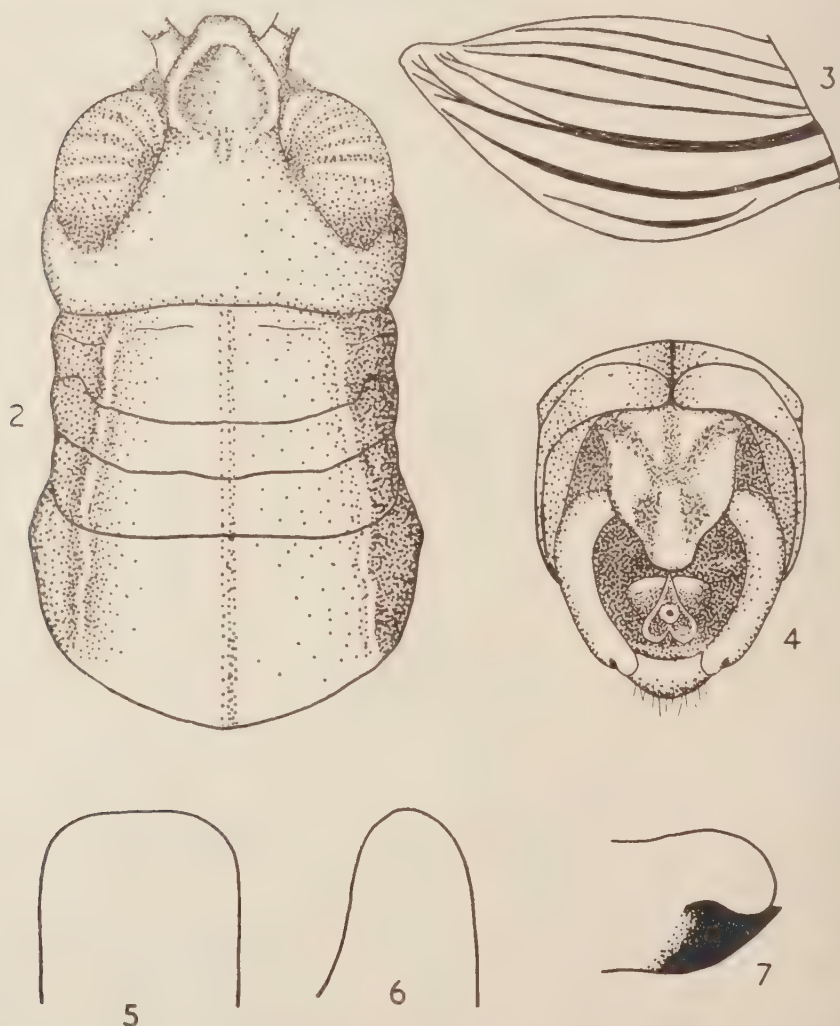
Antenna as long as head and pronotum together. Fastigium of vertex almost as broad as long, semioval, weakly concave and limited by obtuse lateral carinulae. Frontal ridge flat, gradually widening downwards.

Disc of pronotum feebly tectiform; median carina thin, in profile straight; lateral carina well developed and slightly sinuate; posterior margin of metazona broadly obtusangulate, almost rounded. Three sharp transverse sulci. Prosternal tubercle very broad, spade-shaped, with truncate apex and rounded angles. Mesosternal lobe narrow, internally straight, and with straight posterior margin. Mesosternal interspace scarcely broader than long. Metasternal interspace in the male very narrow, the lobes touching each other; in the female as long as broad, the lobes separated.

Elytron short, lobiform, lateral; membrane hard; venation and reticulation thick. Wings rudimentary.

Middle femur with two furrows on the external side. Hind femur moderately wide and thick with weakly serrated upper carina. Hind knee broad, with shallow excision; lower margin of the lower lobe straight. Hind tibia with eight external and nine internal short, thick, obtuse spines. Arolium large, as broad as long.

Apex of the abdomen in the male curved up. Subgenital plate short and broad, with broadly obtusangulate apex. Supraanal plate comparatively short, with sinuate lateral margins and narrowed rounded apex. Cercus regularly incurved, narrow, its upper lobe moderately wide, the lower one ending in a subacute tooth, which is curved inwards and covered by the upper lobe. Subgenital plate in the female



Figs. 2-7.—*Indomerus noxius*, gen. et sp. n. Male. (2) Head and pronotum from above; (3) right elytron; (4) apex of the abdomen from above; (5) prosternal tubercle from behind; (6) prosternal tubercle in profile; (7) apex of the cercus, seen from behind.

broadly obtusangulate. Ovipositor valves short, robust, slightly excurved at the apices ; lower valve with obtusangulate projection on the external side.

Indomerus noxius, **sp. n.**

♂ (Type). Pronotum finely rugulose ; lower margin of the lateral lobe obtusangulate.

Apex of the elytron reaching the middle of the third abdominal tergite ; its anterior margin strongly excurved, apex narrowed and slightly recurved. Wing shorter than elytron, reaching only the second abdominal tergite.

Ratio of length to width of the posterior femur—3.1. Hind knee scarcely reaching the apex of the abdomen.

General coloration ochraceous. Elytron brown ; the scapular and axillary area and the apex ochraceous. Hind femur above with three indistinct dark fasciae and dark internal upper lobe of the knee ; the inside pale yellow ; external side with small dark spots along the lower ridge. Hind tibia pale yellowish, at the base with two dark narrow rings and below it with small lateral spot. Spines and spurs dark apically. Tooth of the cercus black.

♀ (Paratype). As the male, but larger and more robust.

Length of body ♂ 21 mm., ♀ 26 mm. ; pronotum ♂ 5.5 mm., ♀ 7.5 mm. ; elytron ♂ 5.5 mm., ♀ 8 mm. ; hind femur ♂ 11.5 mm., ♀ 15 mm.

INDIA : Til Ajmer, 1.ii.1948, 3 ♂♂ (including type), 6 ♀♀ (*Bhatia*). Damaging wheat.



STUDIES ON AQUEOUS SUSPENSIONS OF INSECTICIDES.

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(PLATE XVII.)

It is becoming increasingly clear that insecticides must be formulated with due regard to the insects to be dealt with, the types of surfaces to which they must be applied, the degree of "weathering" they must withstand, and other local factors that will influence their efficacy.

Laboratory tests have shown that a wettable powder is a most promising type of formulation for application to absorptive mud walls of native-type houses in Africa for the control of adult mosquitos (Hadaway & Barlow, 1949). Surface deposits on mud are greater from water suspensions of wettable powders than from solutions or emulsions.

Studies of the properties of wettable powders have been continued and are described below. Particular attention has been given to the relation between particle size and insecticidal efficiency. These investigations are related primarily to the development of improved formulations for application to absorptive surfaces for adult mosquito control but the fundamentals involved are of interest and importance in other fields.

Materials and Methods.

Test surface.

An absorptive surface which is less variable and less likely to interfere with chemical analysis than mud was needed for general use in testing insecticides as liquids or dispersions. Accordingly plaster of paris was chosen. The ordinary commercial material was not satisfactory but a better type procured from Messrs. British Drug Houses Ltd. was white in colour, varied only slightly from batch to batch and had no free chloride to give high blank values in the determination of chlorinated hydrocarbon insecticides.

The plaster was mixed with water in the proportion of 5 : 2 by weight and allowed to set in moulds consisting of iron or brass rings 7.5 cm. in diameter and 1 cm. deep. They were air dried at 25 C. for at least four days. The final treatment before spraying was to smooth and flatten the surface by scraping with a palette knife. The porosities of the blocks used throughout this work were occasionally checked and found to be 36-37 per cent., changing slightly from one delivery of plaster to another. Within a given batch the variation was much less. Use of less water than the ratio mentioned gave considerably lower porosities.

Spraying tower.

Some of the particles of wettable powders were too large to be sprayed with the usual type of atomising gun. It was necessary, also, to use a dip tube rather than a gravity feed and to spray the whole of a given sample of suspension in order to avoid irregularities due to differential sedimentation of particles of varying size.

A tower of the type described by Webb (1947) was available. The top box containing the window was removed and the square lid screwed centrally over the remaining cylindrical portion, thus giving a tower about 3 ft. high and 10 ins. in diameter. The base was not altered.

The best type of nozzle to use, probably, would have been an air delivery tube containing a centrally placed liquid tube (Potter, 1941) but this required accurate machining in metal, and in order to begin work quickly the simple device used in flit guns was employed. Some commercial powders are so coarse that the liquid delivery tube had to be about 1 mm. in internal diameter to prevent blocking. A glass tube, slightly conical at the free end and placed axially to the tower, delivered compressed air downwards across the end of the dip tube. This dip tube was bent through a right angle so that a small reservoir could be applied to the free end during spraying. The reservoir was the one inch terminal portion of a 3/5 inch test tube fitted with a short length of wide rubber tubing so that it could stand upright. Its capacity was about 3 ml. A truncated glass cone was placed between the lower end of the air tube and the circular hole of 5 ins. diameter in the top of the tower to allow the spray assembly to be far enough from the tower to permit easy access to the dip tube.

A range of air pressures could not be used because the air tube was so wide. The compressor worked at full capacity all the time and could just maintain the required flow of air. The pressure in the air line was about 12 lb. per sq. inch.

Use and calibration of the spraying tower.

The air tube and liquid tube were clamped in various relative positions until a good spray with no spitting was obtained. While a dyed water solution was sprayed, the position of the complete spraying assembly was altered until a good distribution at the base of the tower was arrived at, as judged visually. Uniform distribution occurred over a circle of about 4 ins. diameter in the centre of the tray. This area was large enough to cover the plaster blocks used. After four or five sprayings it was necessary to remove the glass cone at the top of the tower and wipe it. This could be done without disturbing the spray assembly.

If large quantities of commercial wettable powders were to be sprayed, say 200 mg. the required amount was weighed out into the reservoir and dispersed in 2 ml. of distilled water. This suspension was sprayed and any residue in the reservoir or dip tube washed through with a further 1 ml. of water. For smaller amounts a known weight of powder was dispersed in distilled water and made up to 20 ml. One ml. of this suspension was pipetted into the reservoir, using a wide-mouthed pipette, and diluted with 1 ml. of water. After stirring with a thin glass rod the suspension was immediately sprayed, followed by washing as before.

Calibration was performed by catching the deposit from a known weight of sprayed material on a weighed square, 3 × 3 ins. glass plate at the base of the tower. The plate was reweighed after drying. Five determinations were usually made at each dosage applied in order to obtain the ratio between weight sprayed and the dosage obtained in mg. per sq. ft. This ratio did not vary with dosage. Calibration was repeated usually about once a month during the time that the tower was used and variation in the ratio was insignificant.

By careful adjustment of the various parts of the tower, the repeatability of a given dosage was ± 10 per cent. The error could not be made consistently less than this with different materials, although with the same powder successive sprayings often had a much smaller error.

Dosages used in the calibrations were from 25 to 200 mg. per sq. ft., as smaller deposits could not be weighed accurately. It was assumed that the same ratio of weight sprayed to dosage obtained still held good for smaller dosages. Microscopical examination of sprayed plates did not show any obvious variations in frequency of different particle sizes over the sprayed area.

Replicate series of blocks were sprayed with each type of suspension to be investigated for each test. Treated blocks were kept in a cabinet at 25°C. and,

except where otherwise stated, biological tests were carried out 24 hours after spraying.

Insecticides.

Each substance was recrystallised from absolute ethanol. It was necessary to measure the densities for separation by sedimentation in different particle sizes. This was done by a flotation method. The values available in the literature for the density of pp' DDT vary. Gullstrom and Burchfield (1948) use 1.47, Armour Research Foundation (1948) 1.500, and Fankuchen (1946) 1.556. The determination for pp' DDT was repeated, therefore, using both large and small crystals and the same value of 1.55 obtained. This was used in the calculation of sedimentation rates.

The properties of the pure insecticides were :—

			Melting point °C.	Density
DDT.	2,2-bis (p-chlorophenyl) 1,1,1-trichloroethane...	...	109.5	1.55
DDD.	2,2-bis (p-chlorophenyl) 1,1-dichloroethane	110	1.48
Methoxychlor.	2,2-bis (p-methoxyphenyl) 1,1,1-trichloroethane	...	88	1.42
Compound 497.	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene	176-176.5	1.75
γBHC.	Gamma isomer of hexachlorocyclohexane	...	112	1.87

Separation of particles of DDT by sieving.

A ground sample of 5 gm. of DDT was mixed with a 0.1 per cent. solution of Teepol and wet sieved through B.S. Sieves Nos. 110, 150 and 250 in turn, giving the following fractions :—

- Size 0- 60 microns, passing 250.
- 60-100 microns, retained by 250 and passing 150.
- 100-130 microns, retained by 150 and passing 110.
- 130- ∞ microns, retained by 110.

Each fraction was resieved, filtered and dried at 90°C.

Fractionation of insecticides by sedimentation.

The procedure adopted was that of Burchfield (1948). About 10 gm. of each substance were ground in a mortar as finely as possible. With continued grinding, a few ml. of 50 ml. of 1 per cent. Teepol solution in distilled water were added to give a paste and, as more wetting agent was added, a thick cream of insecticide. The cream was washed into a 500 ml. measuring cylinder using the remainder of the 50 ml. of 1 per cent. Teepol and diluted to the 500 ml. mark with distilled water. The series of cylinders used were carefully chosen to have the same length of calibration.

The subsequent operations were as described by Burchfield, the only variation being that the different ranges were separated nine-tenths of the way down the sedimenting column instead of half-way. Each range was purified by sedimenting six or seven times or until the smallest sizes in the range reached the nine-tenths position leaving a perfectly clear liquid above them, thus indicating that no smaller particles were present. All dilutions of the suspensions were made with 0.1 per cent. Teepol in distilled water.

Each purified fraction was transferred to a corked boiling tube and the volume adjusted to 25 ml. One ml. of each of the thoroughly mixed suspensions were taken for analysis. Gamma hexachlorocyclohexane was determined by dehydrohalogenation and all the others by evaporating to dryness in weighed petri dishes, allowance being made for the wetting agent solids. Knowing the concentration in mg./ml. the volumes of the suspensions were adjusted to contain the weight/ml. needed for spraying to give the required dosage in mg./sq. ft. in the spraying tower.

The suspension was mixed thoroughly and 1.0 ml. quickly transferred with a wide-mouthed pipette to the reservoir for application of deposits. One ml. of 0.1 per cent. Teepol was added, the suspension mixed with a thin glass rod and the mixture sprayed. Any residue was washed through with a further 1 ml. of wetting agent solution as described previously. Higher dosages were obtained by concentrating the stock solution, and lower ones by diluting known volumes and taking 1 ml. samples of the diluted mixture.

DDT was separated originally into 0-10, 10-20, 20-40 and 40-80 micron ranges, but later it and all the other insecticides were divided into 0-10, 10-20, 20-40, 40-60 and 60-80 micron ranges. The size ranges given are, of course, Stokes equivalent diameters. It was possible to divide the 0-10 micron range into 0-5 and 5-10 with the heavier substances, γ -BHC and Compound 497. It was considered unnecessary to undertake the additional labour of preparing narrower ranges than those used. It seemed unlikely that there would be any marked difference between two slightly different sizes and the preparation of very narrow size ranges would not be possible commercially.

Crystals of different shapes.

Sixty-micron long needles and 60 \times 15-micron plates were made as described by McIntosh (1947). The plates could not be obtained without a small proportion of smaller rods. Five-micron rods were formed by precipitation from a highly supersaturated alcohol-water mixture. Details of this and similar preparations will be described in a separate report.

Test insect.

The test insects were adult female mosquitos, *Aedes aegypti* (L.), reared at a temperature of 25°C. and relative humidity of 70 to 80 per cent. Eggs are stored for four days on moist filter paper and hatch on the first day in water. The larvae are divided into batches of approximately equal numbers on the third day and are fed on an infusion of dried, ground bread. Pupae begin to appear on the seventh day, and are transferred to baths from which the adults emerge into stock cages. A cage containing 0-1-day-old mosquitos is removed each morning and stored for two days. A guinea pig is then placed in the cage in the early morning. Tests are carried out later in the day with 2-3-day-old female mosquitos that have had one blood meal.

Technique for exposure to treated surface.

Mosquitos were exposed to a treated surface for a short contact time, transferred to a clean cage, and stored for a period of 24 hours. All operations were carried out at a temperature of 25°C. and relative humidity of 70 to 80 per cent. The technique employed was a modification of that devised by Kennedy and Ainsworth (unpublished). The apparatus is illustrated in Pl. XVII, fig. 1.

Female mosquitos are drawn from the stock cage into a wide-mouthed sucking tube and anaesthetised with a current of carbon dioxide passed slowly into the tube. A batch of twenty is tapped lightly on to a thin, circular, bakelite disc and is covered with a perspex funnel 6.5 cm. in diameter. The internal surface of the funnel is polished, and the short stem is provided with a cork and small glass tube. A series

of batches of mosquitos is prepared in this way and left for the short period necessary for the mosquitos to recover and become normally active again. Flight is severely restricted in the confined space under the funnel and the horizontal surface is preferred to the sloping, polished wall of the funnel, so that the mosquitos remain on and walk over the disc or any surface that replaces it.

A disc, bearing mosquitos and covering funnel, is placed over the circular aperture of the platform of the apparatus and is held in position by a trigger mechanism. The surface under test is brought up from below to a position immediately below the disc. When the trigger is released, the bakelite disc is rapidly withdrawn by an attached spring and the funnel drops the short distance on to the test surface. The confined mosquitos leave the surface of the disc at its first movement and resetttle on the test surface. The disc does not come into contact with the test surface at any time and its withdrawal does not interfere with surface deposits.

The test surface is lowered so that it and the mosquitos confined under the funnel can be moved to the bench and the apparatus used for the next batch. At the end of the exposure time the surface and funnel, together, are inverted and carbon dioxide is passed slowly through the glass tube in the cork of the funnel. The anaesthetised mosquitos fall from the surface and while still immobile are transferred from the funnel to the recovery cage. A second check of the number of mosquitos used in the test is made as this is done.

The recovery cages consist of wire gauze cylinders, 3 ins. in diameter and 4 ins. long, with detachable tin-plate ends. The base is lined with white filter paper, and a plug of cotton wool soaked in sugar solution is inserted in the central aperture of the lid. Mosquitos are stored for 24 hours and the numbers dead are then recorded.

Deaths in control batches of mosquitos exposed in exactly the same way to untreated surfaces, or to surfaces treated only with inert dusts, are negligible.

Results.

Treatment of results.

Toxicity tests for the assay of insecticides frequently involve the exposure of batches of insects to different concentrations or dosages of the poison for a constant time. It became evident in the early stages of this work that dosage of aqueous suspensions of crystals was not critical above a certain level. This dosage level was quite low for suspensions of small DDT crystals and accurate application of lower dosages was not possible with the spraying apparatus available. It was decided, therefore, to use a constant dosage for any one test and to vary the period of exposure. There is a straight line relationship between probit kill and logarithm of the time of exposure.

The percentage kill given for each exposure time is a mean of replicate tests carried out on the same day with mosquitos from the same population. All techniques and conditions were standardised as far as possible and replication on the same day was good. Tests were always repeated and, although some day-to-day variations occurred, differences in effectiveness of different formulations were constant.

Commercial wettable powders.

Tests with four samples of commercial 50 per cent. DDT wettable powders indicated an inverse relation between particle size of the insecticide and effectiveness.

The particle size distributions of the DDT portions of the powders were determined by the method of Gullstrom and Burchfield (1948), the DDT content of the pipetted

samples being estimated by dehydrohalogenation, followed by potentiometric determination of the free chloride. The accumulative percentage at each size plotted on log.-probability paper gave a straight line and enabled the 50 per cent. mass size to be found immediately. All the four powders tested gave lines which were parallel and the actual shapes of the distribution curves were therefore similar. Thus it was sufficient to know the 50 per cent. mass size without actually drawing the distribution curves in order to correlate the average particle sizes with different degrees of biological effectiveness.

TABLE I.

Comparison of four commercial DDT wettable powders at a dosage of 25 mg. DDT per sq. ft.

Powder	50 per cent. mass size in microns	Mean percentage kill after exposure of — minutes						
		0.5	1	2	4	8	16	32
A	13	5	40	60	95	100		
B	18	3	35	58	93	100		
C	24	3	23	35	80	100		
D	74				0	10	40	68

The following regression equations relating probit kill to the logarithm of the time of exposure were calculated :—

Powder A. $y = 3.32x + 1.19$. $b = 3.32 \pm 0.41$.

B. $y = 3.41x + 0.96$. $b = 3.41 \pm 0.40$.

C. $y = 3.17x + 0.80$. $b = 3.17 \pm 0.35$.

D. $y = 3.04x - 2.07$. $b = 3.04 \pm 0.46$.

where $x = \log$. (time of exposure in minutes $\times 10$).

DDT crystal size.

A ground sample of DDT crystals was separated by sieving into the four ranges 0-60, 60-100, 100-130 microns and over. Each range was applied at a dosage of 50 mg. DDT per sq. ft. Only crystals in the smallest size range of 0-60 microns were effective against mosquitos exposed to them for periods of eight minutes or less.

TABLE II.

Effectiveness of DDT crystals separated by sieving.

Size range in microns	Mean percentage kill after exposure of — minutes			
	1	2	4	8
0-60	15	45	74	98
60-100	0	0	5	13
100-130	0	0	0	8
over 130	0	0	3	5

DDT crystals were fractionated by sedimentation into substantially pure, narrow limit ranges as described previously, and were tested at a dosage of 25 mg. per sq. ft. There was an optimum size range of 10-20 microns and above this an inverse relation between particle size and effectiveness. Crystals in the 0-10 micron range were less effective than those of 10-20 microns, and reasons for this are discussed later.

TABLE III.

Effectiveness of DDT crystals fractionated by sedimentation.

Crystal size in microns	Mean percentage kill after exposure of — minutes							
	0.25	0.5	1	2	4	8	16	32
0-10		3	18	45	80			
10-20	15	41	70	98				
20-40			5	33	60	85		
40-60				0	0	10	38	
60-80					0	0	3	30

Calculated regression equations are as follows :—

$$0-10 \text{ micron crystals. } y = 3.03x + 0.98. \quad b = 3.03 \pm 0.43.$$

$$10-20 \text{ micron crystals. } y = 3.01x + 2.68. \quad b = 3.01 \pm 0.42.$$

$$20-40 \text{ micron crystals. } y = 2.81x + 0.79. \quad b = 2.81 \pm 0.39.$$

where $x = \log$. (time of exposure in minutes $\times 10$).

The number of particles required to make up a given weight or dosage is inversely proportional to the cube of the radius. A possible explanation of the previous results, therefore, is that the probability of a mosquito acquiring a lethal dose from a large number of small crystals distributed over an area is greater than from a small number of large crystals over the same area.

Plaster blocks were treated with aqueous suspensions of crystals in three different size ranges at different dosages, so that approximately the same number of crystals was present on each block. It was found that crystals in the 10-20 micron range at a dosage of 6 mg. per sq. ft. were more effective than crystals in the 40-80 micron range at a dosage of 400 mg. per sq. ft. The number of crystals, therefore, is of less significance than their size.

TABLE IV.

Effectiveness of equivalent numbers of DDT crystals.

Crystal size in microns	DDT Dosage mg. per sq. ft.	Mean percentage kill after exposure of — minutes			
		1	2	4	8
—	0				3
10-20	6	26	93	100	100
20-40	50	3	45	80	100
40-80	400	3	5	30	60

Microscopical examination of mosquitos after exposure to deposits of DDT crystals on plaster blocks revealed that crystals are removed from the substrate and retained on the tarsi. The number of crystals "picked up" is dependent on the particle size, smaller particles being taken up to a greater extent than larger ones.

Each tarsus of *Aedes aegypti* possesses a pair of claws and no pulvilli and the segments are clothed with black and white scales and hairs. The "pick-up" of crystals is seen more clearly on houseflies, *Musca domestica*, L., and tsetse flies, *Glossina palpalis* (R.-D.), each tarsus of which possesses a pair of claws, two pulvilli and numerous hairs. Large numbers of 10 to 20 micron crystals are removed from the substrate on the fly pulvilli and hairs of the tarsal segments, and the number of crystals removed decreases rapidly as the crystal size increases. The pick-up of DDT crystals on the pulvilli of *G. palpalis* is illustrated in Pl. XVII, fig. 2. Particles

may be picked up on the ventral surface of the abdomen, as well as on the legs. Transference of small crystals from the legs to the antennae, head and other parts of the body takes place during normal cleaning processes. The few larger crystals picked up by the insect are more readily detached and removed from its body during cleaning and other movements than are smaller particles.

Tests have shown that there is an inverse relation between particle size of DDT and effectiveness against tsetse flies as well as against mosquitos. Differences in effectiveness of crystals can be explained, therefore, by differences in the amounts of insecticide removed from the surface and retained by mosquitos and flies.

Mosquitos exposed individually for short contact times to a treated surface in 2 × 1 inch glass tubes tend to remain stationary. On the other hand, mosquitos exposed by the funnel technique walk over the treated surface. Exposures of mosquitos for a given time by the latter method result in the pick-up of more crystals and in higher kills than exposures for the same time by the first method. Busvine and Barnes (1947) have shown that the percentage mortality among bed bugs kept in motion for a specified period over a given deposit of DDT is greater than that among stationary bed bugs exposed to the same deposit for the same time.

DDT crystal shape.

Aqueous suspensions of DDT rods 5 microns in length, of fine needle crystals 60 microns in length, and of 60 × 15 micron plates were prepared as described previously and compared with suspensions of ground crystals separated into size ranges by sedimentation to determine the effect of particle shape on toxicity. It was not possible to use needles longer than 60 microns because of the risk of breakage during spraying operations.

The 5 micron rods and crystals in the 0-10 micron range were equally effective on plaster at a dosage of 25 mg. per sq. ft. The needles were slightly more effective than crystals in the 10-20 micron range and these, in turn, were more effective than the 60 × 15 micron plates.

The dimensions of the crystals were such that the weights of the 10-20 micron crystals and the plates were approximately of the same order and both were heavier than the needles. Crystal length alone, at least up to 60 microns, is not critical and it is probable that shape and mass of crystals together influence pick-up and determine their effectiveness.

TABLE V.
Effectiveness of DDT crystals of different shapes.

Crystal type	Mean percentage kill after exposure of — minutes				
	0.5	1	2	4	8
0-10 μ	5	13	35	65	
5 μ rods	3	8	40	60	
10-20 μ	26	67	100		
60 μ needles	45	93	100		
60 × 15 μ plates	15	43	63	93	
60-80 μ				0	0

Residual toxicity of DDT crystals.

The residual toxicities of crystals in three different size ranges were compared by exposing mosquitos to plaster blocks 1 day, 8 weeks and 21 weeks after treatment at a dosage of 50 mgs. DDT per sq. ft. Exposures after each time interval were made on the same triplicate series of blocks which were kept at 25°C. throughout the test.

The blocks treated with 10-20 micron crystals showed a slight increase in effectiveness over the 21 week period ; those treated with 20-40 micron crystals were less effective after 21 weeks than they were 1 day and 8 weeks after spraying, and those treated with 40-80 micron crystals already showed a loss of effectiveness at the end of 8 weeks.

Microscopical examination of the blocks after the last exposures of mosquitos showed the presence of numerous 10-20 micron crystals, few 20-40 micron crystals and only occasional 40-80 micron crystals. Differences in residual toxicity of the three size ranges were due to differences in the rates at which the surfaces were depleted of crystals and not to a greater loss of toxicity of DDT in large crystal form than in small crystal form. Although the pick-up of large crystals is much less than that of small ones, some large crystals are removed from the surface by each batch of mosquitos. At a given dosage, the number of large crystals is very much less than that of small ones and a plaster surface is more rapidly depleted of large crystals than of small ones.

TABLE VI.
Residual toxicity of DDT crystals.

Size range in microns	Age of deposit	Mean percentage kill after exposure of — minutes					
		0.5	1	2	4	8	16
10-20	1 day	8	32	83	97		
"	8 weeks	17	52	98	100		
"	21 weeks	22	65	100	100		
20-40	1 day		7	32	75	100	
"	8 weeks		10	48	88	100	
"	21 weeks		0	2	18	37	
40-80	1 day			5	15	43	75
"	8 weeks			2	5	21	34
"	21 weeks			0	0	0	3

Dosage of DDT crystals.

DDT crystals in the size range of 10-20 microns were applied to plaster blocks at dosages ranging from 1 to 50 mg. per sq. ft. It was found that there was no significant increase in kills of mosquitos exposed to dosages above 3 mg. per sq. ft. for contact periods of two minutes. There is a limit to the number of crystals picked up during a given contact period and at dosages above 3 mg. per sq. ft. the number of 10-20 micron crystals is excessive, so that pick-up and kill are independent of dosage.

TABLE VII.
Effectiveness of 10-20 micron DDT crystals at different dosages.

	Dosage in mg. DDT per sq. ft.					
	1	3	6	12	25	50
Mean percentage kill after exposure of 1 min. ...	15	30	30	35	28	43
Mean percentage kill after exposure of 2 mins. ...	53	78	83	80	73	83

Dosage, however, influences the residual toxicity of the deposits. Each batch of mosquitos exposed to a treated block removes a proportion of the crystals and, if

successive batches are exposed to the same block, the surface is gradually depleted of crystals.

Successive batches of 20 mosquitos were exposed for two minutes each to blocks treated with 10-20 micron crystals at dosages of 1, 3 and 6 mg. per sq. ft. The number of batches required to deplete the surface of crystals increased as the dosage increased. When kills had been reduced to zero, further batches of mosquitos were exposed to the blocks for longer periods of half an hour or more and no deaths occurred.

TABLE VIII.
Persistence of deposits of 10-20 micron DDT crystals.

Dosage mg. per sq. ft.	Mean percentage kill after exposure of 2 minutes										
	Batch										
	1	2	3	4	5	6	7	8	9	10	11
1	45	23	8	10	3	0	0				
3	98	98	90	73	83	75	60	35	18	8	0
6	100	98	100	95	95	85	78	73	73	70	68

0-10 micron DDT crystals.

Results of previous tests have shown that ground crystals in the 0-10 micron size range and 5 micron rods are less effective than crystals in the 10-20 micron range on plaster blocks.

A similar difference was obtained when aqueous suspensions of two commercial samples of 50 per cent. DDT pastes were applied to plaster blocks at a dosage of 25 mg. per sq. ft. The sample containing crystals with a mean length of 5-6 microns was less effective than that containing crystals with a mean length of 10-12 microns.

TABLE IX.
Effectiveness of two samples of DDT pastes on plaster blocks.

Sample	Mean crystal size in microns	Mean percentage kill after exposure of — minutes			
		0.5	1	2	4
I	5-6	8	20	48	73
II	10-12	48	83	100	100

Comparisons on mud blocks and compressed fibreboards show that crystals in the 0-10 micron range can be at least as effective as those in the 10-20 micron range. Differences in effectiveness on the three surfaces are probably due to differences in availability on surfaces of different structure.

It is difficult to account for the different results obtained with the two absorptive surfaces, mud and plaster. They may be explained partially by differences in surface morphology which affect the degree of contact between the crystals and the mosquito tarsi. Whereas the surface of a plaster block contains innumerable depressions smaller than the 10-20 micron crystals that of a mud block is more irregular and the depressions generally are much larger than both the 10-20 micron crystals and the width of the mosquito tarsal segments. The width of the last tarsal segment of *Aedes aegypti* is approximately 60-80 microns. It has been shown, however, that

dosages of 10–20 micron crystals as low as 3 mg. per sq. ft. are as effective as a dosage of 25 mg. per sq. ft. For differences in surface morphology to account entirely for the different results obtained with 0–10 micron crystals on mud and plaster it would be necessary, therefore, for the majority of the crystals to be rendered ineffective by deposition in the surface depressions of the plaster blocks. It is unlikely that this is the case and other factors are probably also involved. For instance, there may be greater adhesion of the 0–10 micron crystals to plaster than to mud.

The spray droplets do not wet and penetrate the surface of the hydrophobic fibre board and, after drying, the 0–10 micron crystals are just as available as the 10–20 micron crystals.

TABLE X.

Effectiveness of DDT crystals on mud-blocks and fibre boards.

Surface	Crystal size in microns	DDT dosage per sq. ft.	Mean percentage kill after exposure of — minutes					
			0.5	1	2	4	8	16
Mud	...	0–10	41	83	100	100		
"	...	10–20	10	58	90	100		
"	...	20–40		0	3	8	28	
Fibreboard	...	0–10		13	45	80	100	
"	...	10–20		8	31	73	100	
"	...	20–40				0	3	13

Other insecticides.

Pick-up of DDT crystals by mosquitos from a plaster surface is dependent on particle size. A similar inverse relation between particle size and effectiveness should occur, therefore, with other solid insecticides.

Results given in Tables XI to XVI of tests with aqueous suspensions of ground crystals of methoxychlor, DDD, Compound 497 and γ -BHC on plaster blocks show that this is so. The optimum particle size of 10–20 microns is the same for methoxychlor and DDD as it is for DDT. Crystals of these two compounds in the 0–10 micron range are less effective than those in the 10–20 micron range for the same reasons, probably, as for DDT. Particle size of both Compound 497 and γ -BHC does influence effectiveness, but to a lesser extent than with DDT. Both of these compounds are intrinsically more toxic than DDT to mosquitos, and the pick-up of only a few large crystals may be lethal.

The relation between particle size of γ -BHC and effectiveness is of further interest from the point of view of persistence. Loss by vaporisation means a reduction in particle size and variation in the residual toxicity of particles of different initial sizes. Investigations are complicated by the fact that, at the same dosage, the number of particles decreases rapidly as the size increases and that repeated exposures at regular time intervals to the same treated blocks result in the gradual depletion of crystals. It is necessary, therefore, to treat large numbers of blocks and expose mosquitos to a new series after each time interval to obtain a true picture of the persistence of particles in the different size ranges. Tests carried out so far show that toxicity of the smallest particles falls rapidly and indications are that a wide range of particle size of γ -BHC is necessary to attain maximum efficiency for both initial and residual toxicity.

TABLE XI.

Effectiveness of methoxychlor crystals at dosage of 25 mg. per sq. ft.

Particle size in microns	Mean percentage kill after exposure of — minutes				
	8	16	32	64	128
0-10	0	3	40	60	
10-20	0	28	70	100	
20-40		0	3	20	73
40-60		0	0	5	8

TABLE XII.

Effectiveness of DDD crystals at dosage of 25 mg. per sq. ft.

Particle size in microns	Mean percentage kill after exposure of — minutes				
	2	4	8	16	32
0-10	0	3	24	79	
10-20	33	73	100	100	
20-40	0	18	70	95	
40-60		0	5	23	38
60-80			0	8	18
Untreated					3

TABLE XIII.

Effectiveness of equivalent numbers of DDD crystals.

Particle size in microns	Dosage in mg./sq. ft.	Mean percentage kill after exposure of — minutes			
		2	4	8	16
10-20	5	23	63	93	100
20-40	38	18	44	83	100
40-60	175	0	0	13	45

TABLE XIV.

Effectiveness of crystals of Compound 497 at a dosage of 25 mg. per sq. ft.

Particle size in microns	Mean percentage kill after exposure of 15 seconds
0-5	100
5-10	100
10-20	100
20-40	100
40-60	79
60-80	3

TABLE XV.

Effectiveness of equivalent numbers of crystals of Compound 497.

Particle size in microns	Dosage in mg./sq. ft.	Mean percentage kill after exposure of		
		15 secs.	30 secs.	1 minute
20-40	6	58	80	93
40-60	36	73	95	100
60-80	79	28	58	73
20-40	12	88		
40-60	72	100		
60-80	158	75		

TABLE XVI.

Effectiveness of crystals of γ -BHC at dosage of 25 mg. per sq. ft.

Particle size in microns	Mean percentage kill after exposure of 15 seconds
0-5	48
5-10	85
10-20	90
20-40	97
40-60	65
60-80	63

Inert diluents.

It is necessary when preparing wettable powders of commercial DDT to add a certain amount of inert ingredient to improve the grinding and keeping properties and, in some instances, to serve as an extender. The properties of the inert materials used in wettable powders vary considerably. Tests were carried out, therefore, to investigate their effects on the toxicity of the insecticide.

Three commercial wettable powders differing only in the relative amounts of insecticide and inert diluent were compared at a dosage of 50 mg. DDT per sq. ft. The DDT contents of the powders were 20, 33 and 50 per cent. Laboratory prepared suspensions containing different proportions of speswhite kaolin to 20-40 micron DDT crystals were also compared.

Results show that there is a masking of the insecticide by the diluent and that this effect is intensified as the proportion of diluent to insecticide increases. The probability of a mosquito picking up particles of DDT decreases as the ratio of inert particles to insecticide particles increases.

TABLE XVII.

Comparison of three commercial wettable powders.

DDT content of wettable powder	DDT dosage mg. per sq. ft.	Mean percentage kill after exposure of — minutes		
		4	8	16
20 per cent.	50	8	33	98
33 " "	50	30	58	100
50 " "	50	45	90	100

TABLE XVIII.

Effect of increasing proportion of diluent to 20-40 micron DDT crystals.

Dosage in mg. per sq. ft.		Mean percentage kill after exposure of — minutes				
20-40 μ DDT	Speswhite	1	2	4	8	16
50	0	15	65	93	100	
50	50	13	45	58	93	
50	150		15	20	48	95
50	950		3	8	18	55
0	50					0

Particle size of diluent.

Aqueous suspensions of mixtures of DDT crystals and three samples of slate dusts with different ranges of particle size were applied to plaster blocks at a dosage of 50 mg. DDT per sq. ft. and 50 mg. slate dust per sq. ft. Details of the particle sizes of the slate dusts are as follows :—

Sample	Percentage less than — microns				
	5	10	20	40	104
1. 10 μ	82	93	99	100	—
2. 95 per cent. passing 200 B.S. Sieve	21.5	43	62	80	98
3. 75 per cent. passing 200 B.S. Sieve	17	34	49	62	84

This variation in the range of particle size of the slate dust had no significant influence on the effectiveness of the DDT crystals.

TABLE XIX.

Effect of particle size of slate dust on toxicity of DDT crystals.

DDT crystal size in microns	Slate dust sample	Mean percentage kill after exposure of — minutes			
		1	2	4	8
10-20	—	75	90	100	
10-20	1. 10 μ	55	78	100	
10-20	2. 95 per cent. passing 200 B.S.S.	45	78	90	
10-20	3. 75 per cent. passing 200 B.S.S.	51	70	85	
—	1. 10 μ			0	
20-40	—	23	70	98	100
20-40	1. 10 μ	23	65	98	100
20-40	2. 95 per cent. passing 200 B.S.S.	10	55	93	100
20-40	3. 75 per cent. passing 200 B.S.S.	8	55	95	100
—	1. 10 μ				5

Type of diluent.

Aqueous suspensions of DDT crystals and different types of diluent were applied to plaster blocks at a dosage of 50 mg. DDT per sq. ft. and 50 mg. diluent per sq. ft.

Deposits from suspensions of DDT crystals and fulbent gave consistently low kills. Fulbent 150 has the property of swelling when wetted, and gel formation may interfere with the availability of the insecticide particles. Variation in the degree of masking of the insecticide by the other diluents occurred but was not great and differences in effectiveness produced by these diluents were small in comparison with those due to particle size of the insecticide.

Results of some tests are as follows :—

TABLE XX.
Effect of different diluents on toxicity of DDT crystals.

DDT crystal size in microns	Diluent	Mean percentage kill after exposure of	
		2 mins.	4 mins.
10-20	—	93	
10-20	Kenya diatomite ...	88	
10-20	Stockalite kaolin ...	73	
10-20	Speswhite kaolin ...	80	
10-20	Slate dust, RF 10 μ	80	
10-20	Fulbent 150 ...	35	
20-40	—		100
20-40	Kenya diatomite ...		88
20-40	Stockalite kaolin ...		63
20-40	Speswhite kaolin ...		64
20-40	Slate dust RF 10 μ		90
20-40	Fulbent 150 ...		53
20-40	Talc ...		77

Initial and residual toxicities of deposits on plaster blocks from four 50 per cent. BHC wettable powders containing different diluents, diatomite, chalk, talc, and fuller's earth, were very similar.

Wetting agents.

Deposits of DDT crystals from aqueous suspensions on absorptive plaster blocks were compared using two different wetting agents, Teepol and goulac (cellulose sulphite lye). It is necessary to use goulac at higher concentrations than Teepol. There was no difference in effectiveness when Teepol at a concentration of 4 per cent. and goulac at a concentration of 20 per cent. of the weight of the DDT were used.

When an aqueous suspension of DDT crystals is applied to hydrophilic absorbent materials such as mud and plaster, the wetting agent solution is absorbed and the insecticidal particles remain on the surface and are readily available for pick-up. The presence of a wetting agent is, however, more important when the suspension is applied to a non-absorbent material like glass because the wetting agent solution is not absorbed and, on drying, it causes the crystals to adhere strongly to the surface. The same crystals are, therefore, less effective on glass plates than on plaster blocks. This was shown to be the case with deposits of 50 mg. DDT per sq. ft. from a 50 per cent. DDT wettable powder.

TABLE XXI.

Comparison of DDT wettable powder on glass and plaster.

Surface	Mean percentage kill after exposure of — minutes				
	2	4	8	16	32
Glass		13	42	62	83
Plaster	22	48	70	98	

The effectiveness of 10-20 micron DDT crystals varied considerably on a number of different surfaces. Adhesion of crystals similar to that on glass, due to the presence of wetting agent solids after drying, also occurs on other surfaces. In addition, the droplets of a suspension remain distinct on hydrophobic absorptive surfaces and crystals aggregate as the wetting agent solution dries instead of remaining as discrete particles, as on plaster. Again, the same crystals are less effective on compressed fibreboard than on plaster. Hairs, fibres and other surface irregularities may also affect the availability of deposits and crystals which have penetrated below them.

TABLE XXII.

Effectiveness of 10-20 micron DDT crystals on different substrates.

Surface	DDT dosage mg. per sq. ft.	Mean percentage kill after exposure of — minutes						
		0.5	1	2	4	8	16	32
Plaster ...	25	43	60	93	100			
Mud ...	25	33	55	82	100			
Fibreboard ...	25		29	68	90	100		
Limewash ...	50	100	100					
Glass ...	50		8	35	78	100		
Veneered Wood	50			10	40	93	100	
Poplin ...	50			40	65	100	100	
Loose texture wall board...	50				0	3	45	98

Discussion.

Smith and Goodhue (1942) gave a review of literature on particle size in relation to insecticidal efficiency and concluded that, in general, the smaller particles of solid insecticides are the more effective. Most of the references relate to experiments with stomach poisons. Smith (1936) ground samples of pyrethrum powder for different periods and tested them in water suspension. The finest powder paralysed fourth-instar larvae of mosquitos, *Culex quinquefasciatus* Say, more rapidly than coarser preparations, and was most effective against *Aphis rumicis* L. McGovran, Cassil and Mayer (1940) prepared narrow limit fractions of paris green and determined their effects as stomach insecticides against adults and larvae of the Mexican bean beetle, *Epilachna varivestis* Muls. Mortalities progressively increased as the average diameter of particles decreased from 22 to 12 to 1.1 microns.

There are few references to the relation between particle size and toxicity of the newer, synthetic, contact insecticides. Häfliger (1949) fed formulations of DDT separately to honeybees and found that toxicity increased with decreasing particle size. Woodruff and Turner (1947) prepared mixtures of DDT and diatomaceous earths by three methods of processing. The range of particle size produced by each method was large and there was considerable overlap of the three ranges. Reduction

of DDT particle size caused an increase in toxicity of the residues from water suspensions of these mixtures to house-flies, *Musca domestica* L.

McIntosh (1947) made a detailed study of the effects of crystal size and shape on contact toxicity of simple aqueous DDT suspensions using a dipping technique with *Tribolium castaneum* (Hbst.) as test insect. He found that the suspension types could be arranged in order of decreasing toxicity, thus :—

Needle aggregates	(c. 400 μ).	} identical toxicity.
Short acetone needles	(c. 120 μ).	
Plate aggregates	(c. 240 \times 140 μ).	
Short alcohol needles	(c. 40 μ).	} identical toxicity.
Plates	(60 \times 15 μ).	
Colloidal suspension		

It was shown that the toxicity of DDT suspensions increased with increasing size of crystal in suspension, and that these differences in toxicity were paralleled by retention of greater amounts of poison from coarser than from finer suspensions.

McIntosh (1949) found a similar increase in toxicity with increase in particle size of DDT, but an inverse relation of toxicity to the particle size of rotenone in subsequent experiments with *Oryzaephilus surinamensis* (L.). Large hexagonal plate-shaped crystals of rotenone (mean size = 157 \cdot 118 μ) were less toxic than small hexagons (mean size = 21 \times 13 μ).

In experiments described in this paper mosquitos were exposed for short contact periods to plaster blocks treated with aqueous suspensions of ground crystals separated into different size ranges by sedimentation. The insecticide particles were readily available to the insects on a plaster surface and were in a similar condition to those in commercial powders. They were more or less rounded and therefore, unlike long thin needles, not liable to be broken by spraying or by movements of the insects. The sedimentation method used gave ranges of sizes rather than specific ones, but these ranges were pure within the limits given. DDT crystals grown by precipitation from solution always contain varying small percentages of sizes other than the one required. It is important to ensure that the size or size ranges used are essentially pure, for a given preparation may owe almost the whole of its activity to a comparatively small percentage of effective particles.

The results of these experiments show that there is an optimum crystal size of 10–20 microns on plaster blocks and that above this there is an inverse relation between particle size and effectiveness. On mud blocks the inverse relation is continuous and crystals in the 0–10 micron range are the most effective. It is thought that this difference is due to the nature of the plaster surface which interferes, to some extent, with the availability of the very small particles. Crystals in the optimum size range were more effective than larger crystals even when equivalent numbers, and therefore different dosages, were compared.

Differences in effectiveness of crystal size ranges are attributed to differences in amounts of insecticide removed from the substrate by and retained on the insect. Pick-up of large crystals is much less than that of smaller ones, and, in addition, the few large crystals that are taken up are subsequently more readily detached from the insect by cleaning and other movements. McIntosh found that retention of DDT by *Tribolium castaneum* after complete immersion is greater from coarser than from finer suspensions.

Crystal length alone is not critical, for fine 60 micron needles are slightly more effective than ground crystals in the 10–20 micron size range. It is probable that crystal shape and mass together determine the degree of pick-up and effectiveness.

The inverse relationship between particle size and effectiveness is not dependent on any property of DDT and is not peculiar to this insecticide. It applies also to

ground crystals of methoxychlor and DDD. The effectiveness of Compound 497 and of γ -BHC is influenced less by particle size than is that of DDT. These compounds are essentially more toxic than DDT to mosquitos and the pick-up of only a few large crystals may be lethal, or penetration of these insecticides through the insect cuticle may be so rapid that retention of particles on the insect body is unnecessary.

Practical applications of these results are obvious. Considerable improvement in the efficiency of DDT wettable powders can be achieved by limitation and reduction of the size of the DDT particles to the optimum of 10 to 20 microns. Particles of this size are easily suspended, and even at quite low dosages are present in sufficient numbers to provide effective and persistent deposits. On the other hand, it may be necessary to include a wider range of particle sizes in BHC wettable powders to obtain maximum efficiency for both initial and residual toxicity.

Further improvement can be achieved by the inclusion of a minimum amount of inert diluent to reduce its masking effect. At a given dosage of DDT, deposits from a 50 per cent. wettable powder are more effective than those from wettable powders containing lower percentages of insecticide. McCauley, Fay and Simmons (1948) found that a DDT suspension from a 90 per cent. water-wettable powder was more effective than a DDT suspension from a 50 per cent. water-wettable powder against *Anopheles quadrimaculatus* Say, in rooms treated at a dosage of 200 mg. DDT per sq. ft.

Kennedy (1947) first showed that mosquitos are activated and excited by sub-lethal DDT doses and that, when excited by DDT, they move preferentially toward light. Muirhead Thomson (1950) carried out experiments in native houses in Tanganyika and showed that the irritant effect of surfaces treated with a DDT dispersible powder drives *Anopheles gambiae* Giles from the shelter of the house. The number of dead mosquitos on the floor sheet was only a fraction of those escaping unharmed into window traps. On the other hand, numbers of dead *A. gambiae* were taken on the floor sheet of a house treated with "Gammexane" dispersible powder, and the window-trap catch was low and all Anophelines found there were already dead. Improvement of the DDT wettable powder by reduction of the particle size of the insecticide may overcome the disadvantage of its irritant effect on mosquitos. The use of the optimum size of DDT particles should result in the "pick-up" of a lethal dose after only brief contact with a treated surface and before the irritant effect has driven the mosquito from the house.

It is difficult to compare tests of insecticides by various authors when different formulations and substrates have been used. Standardization of both formulation and substrate is essential in comparative tests. Availability of the insecticide is a most important factor in the insect-insecticide relationship and it is influenced considerably by the nature of the surface. For instance, when an aqueous suspension of crystals is applied to plaster the wetting agent solution is absorbed and the crystals are readily available for pick-up on the surface. The same crystals are less effective on glass because the wetting agent solution is not absorbed and, on drying, it causes the crystals to adhere strongly to the surface.

The inverse relation between particle size and effectiveness is not necessarily true for crystals that grow on surfaces treated with DDT solutions. Crystals of DDT grown from solvents on surfaces vary in their orientation with regard to the plane of the surface as well as in their size and degree of adhesion. Long, fragile needles may easily be more toxic than smaller crystals if it is possible for the insect to break off small pieces. On fibrous material it is common for DDT to crystallise as needles with only one end attached to the fibre and the other projecting away. These crystals will be more available to an alighting insect than crystals lying flat on a surface.

Deposits from a given formulation are often more toxic to one insect species than to another. An important factor contributing to this difference is the amount of insecticide available for penetration through the insect cuticle to the site of action,

and this depends to a large extent on the behaviour of the insect during the contact period, its stance, and the morphology of the tarsi. The amount of insecticide picked up by the insect depends, for example, on whether it walks over the treated surface or merely alights on it and then remains stationary, on the number of tarsal segments making contact with the surface, on the presence or absence of pulvilli, and on the hairiness of the tarsal segments.

Summary.

Mosquitos were exposed for short contact periods to deposits on plaster blocks from aqueous suspensions of insecticides.

There is an inverse relation between the size of ground DDT crystals fractionated into size ranges by sedimentation and their effectiveness. Smaller particles are more effective than larger ones even when equivalent numbers are compared. Differences in effectiveness are attributed to differences in the amounts of insecticide picked up and retained by the insect.

There is no significant increase in kills of mosquitos exposed for the same contact period to dosages of 10-20 micron DDT crystals above 3 mg. per sq. ft. "Persistence" of deposits of these crystals, however, increases as the dosage increases.

Crystal length alone is not critical, for fine 60 micron DDT needles are slightly more effective than ground crystals in the 10-20 micron range. The shape and mass of crystals together influence pick-up and determine their effectiveness.

There is also an inverse relation between crystal size and effectiveness of methoxychlor and DDD. The effectiveness of Compound 497 and of the gamma isomer of benzene hexachloride is influenced much less by particle size than is that of DDT.

The inert ingredient of a wettable powder has a masking effect on the insecticide and this is intensified as the proportion of diluent to insecticide increases.

The availability of the deposit from an aqueous suspension of DDT crystals depends largely on the nature of the surface to which it is applied.

Acknowledgements.

We wish to acknowledge with thanks the supply of samples of DDT from the Geigy Company Ltd., the Murphy Chemical Company Ltd., and the Shell Petroleum Company Ltd., of gamma isomer of benzene hexachloride from Imperial Chemical Industries Ltd.; and of Compound 497 from Julius Hyman & Company, U.S.A. Our thanks are due, also, to the Delabole Slate Co. Ltd., the English China Clay Sales Co., Dalgety and Co. Ltd., and Fuller's Earth Union Ltd. for the supply of samples of inert dusts.

References.

- ARMOUR RESEARCH FOUNDATION. (1948). *Analyt. Chem.*, **20**, p. 275.
BURCHFIELD, H. P. (1948). *Analyt. Chem.*, **20**, p. 1168.
BUSVINE, J. R. & BARNES, S. (1947). *Bull. ent. Res.*, **38**, p. 81.
FANKUCHEN, I. & others. (1946). *Science*, **103**, p. 25.
GULLSTROM, D. K. & BURCHFIELD, H. P. (1948). *Analyt. Chem.*, **20**, p. 1174.
HADAWAY, A. B. & BARLOW, F. (1949). *Bull. ent. Res.*, **40**, p. 323.
HÄFLIGER, E. (1949). *J. econ. Ent.*, **42**, p. 523.
KENNEDY, J. S. (1947). *Bull. ent. Res.*, **37**, p. 593.

- MCCAULEY, R. H., FAY, R. W. & SIMMONS, S. W. (1948). *J. nat. Malar. Soc.*, **7**, p. 294.
- MCGOVAN, E. R., CASSIL, C. C. & MAYER, E. L. (1940). *J. econ. Ent.*, **33**, p. 525.
- MCINTOSH, A. H. (1947). *Ann. appl. Biol.*, **34**, p. 586.
- MCINTOSH, A. H. (1949). *Ann. appl. Biol.*, **36**, p. 535.
- MUIRHEAD THOMSON, R. C. (1950). *Trans. R. Soc. trop. Med. Hyg.*, **43**, p. 401.
- POTTER, C. (1941). *Ann. appl. Biol.*, **28**, p. 142.
- SMITH, C. L. (1936). *J. N.Y. ent. Soc.*, **44**, p. 317.
- SMITH, C. M. & GOODHUE, L. D. (1942). *Industr. Engng Chem.*, **34**, p. 490.
- WEBB, J. E. (1947). *Bull. ent. Res.*, **38**, p. 209.
- WOODRUFF, N. & TURNER, N. (1947). *J. econ. Ent.*, **40**, p. 206.
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FIG. 1. Apparatus for exposing mosquitos to treated plaster blocks.

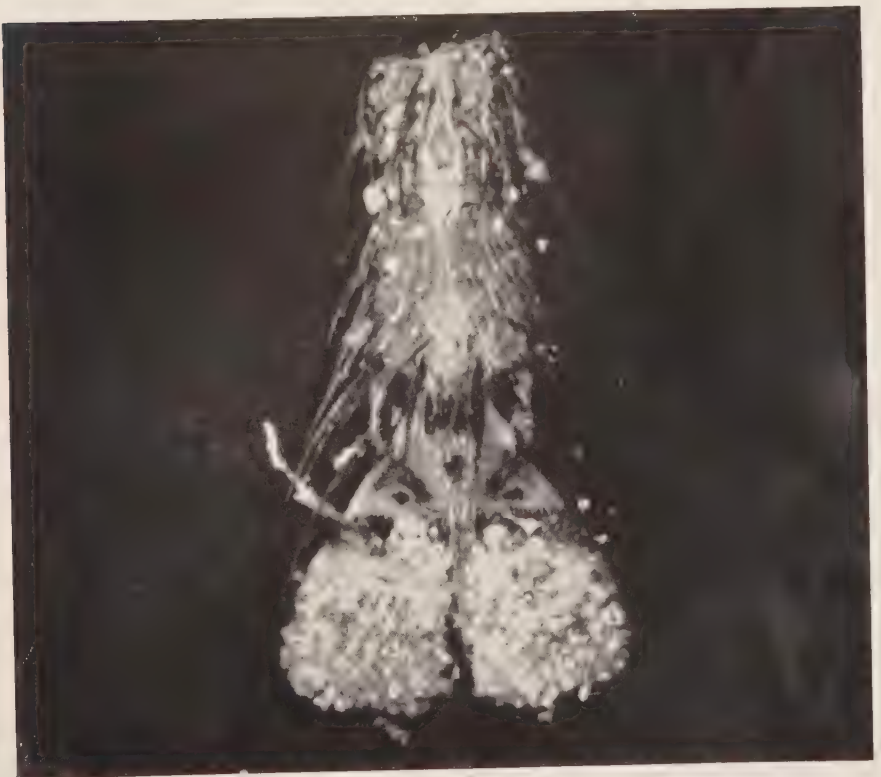


FIG. 2. Pulvilli and last tarsal segments of *Glossina palpilis* showing 10-20 micron DDT crystals picked up after 30 seconds contact with plaster blocks treated at a dosage of 25 mg. per sq. ft.

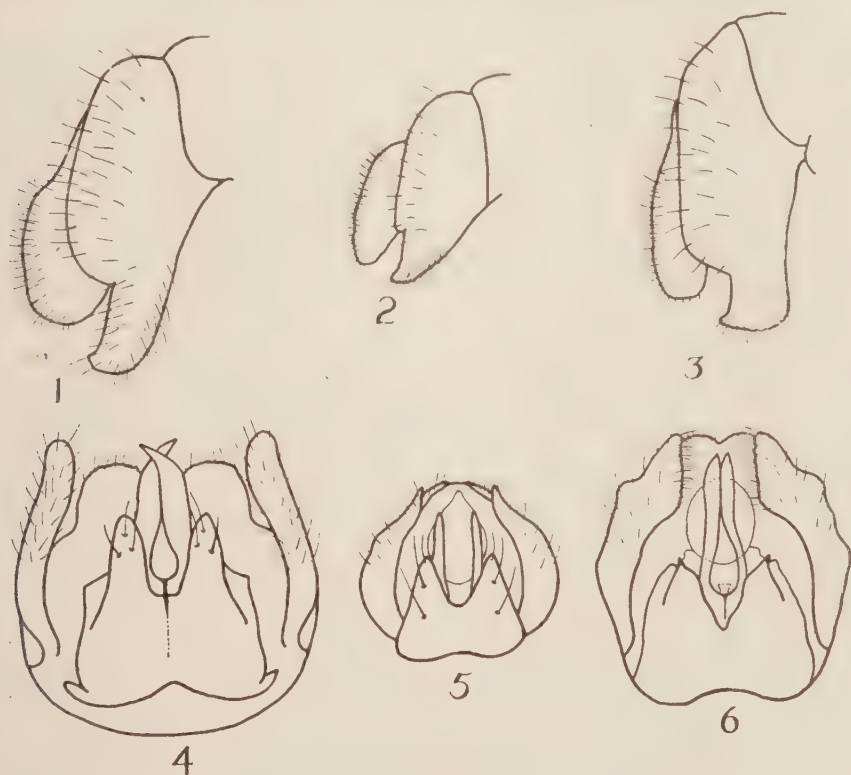
TWO NEW SPECIES OF *PSEUDIASTATA* (DIPT., DROSOPHILIDAE) PREDACIOUS ON THE PINEAPPLE MEALYBUG.

By Curtis W. SABROSKY.

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 United States Department of Agriculture.*

The genus *Pseudiasata* was described by Coquillett (1908) for a single species from Maryland, *P. nebulosa*, which he placed in the family GEOMYZIDAE. Melander (1913b) referred it to the DROSOPHILIDAE and he has been followed in this by subsequent workers. The convergent anterior frontal bristles are suggestive of MILICHIIDAE, however, and the genus may run to that family in some keys.

For many years, the genus was known only from the holotype of *nebulosa* and another specimen from Panama (Sturtevant, 1921). Subsequently, a species identified as *nebulosa* was investigated in Central America and introduced into Hawaii as a biological control against the pineapple mealybug, *Pseudococcus brevipes* (Ckll.) (Fullaway, 1933; Carter, 1935). In 1937, Costa Lima described a second species as *Pseudiasata brasiliensis*, also predacious on *Pseudococcus brevipes*.



Figs. 1-3.—Lateral aspect of male terminalia: (1) *P. nebulosa*; (2) *P. vorax*; (3) *P. pseudococcivora*.

Figs. 4-6.—Anterior aspect of male terminalia: (4) *P. nebulosa*; (5) *P. vorax*; (6) *P. pseudococcivora*.

Recently a series from Trinidad was submitted for study through the kindness of Dr. F. van Emden of the Commonwealth Institute of Entomology, and a critical review of this material and of other available examples in conjunction with the type of *nebulosa* revealed the existence of two undescribed species. Both of the latter species were also reared from larvae predacious on the pineapple mealybug, *Pseudococcus brevipes*. The holotype of *Pseudiasata nebulosa* was collected at light, and the larval habit of this species (s. str.) has never been discovered.

Adults of the four species before me are virtually indistinguishable on superficial characters. Differences in the wing markings are apparently only variations in extent and intensity, and the fundamental pattern of all four species is the same (fig. 7). Males can be separated by comparison of the lateral and anterior aspects of the terminalia (figs. 1-6), but females can be identified only by association with males, as far as can be determined at present. *Pseudiasata brasiliensis*, known to me only from the description, is easily distinguished by the wing pattern (fig. 8).

***Pseudiasata* Coquillett.**

Coquillett, 1908, Proc. ent. Soc. Wash., **9**, p. 148; Melander, 1913, J.N.Y. ent. Soc., **21**, p. 289; Melander, 1913, Psyche, **20**, p. 167; Sturtevant, 1921, Publ. Carnegie Instn, no. 301, p. 55; Malloch & McAtee, 1924, Proc. biol. Soc. Wash., **37**, pp. 30-31; Duda, 1924, Arch. Naturges., (A) **90** (3) pp. 175, 178.

Eye bare, large, occupying most of head in profile, the cheek linear; front with numerous short hairs; occiput decidedly concave; arista microscopically pubescent; thorax densely covered with hairs not arranged in definite rows; scutellum (except for bristles), mesopleuron, and pteropleuron bare; legs short, at least some tibiae with preapical dorsal bristles; wing pictured, venation and colour pattern as figured (fig. 7), costa fractured twice, with a strong bristle before each break, costa extending to fourth vein, discal and second basal cells confluent, anal cell present but small. Chaetotaxy: 1 inner and 1 outer vertical, cruciate postverticals (short), cruciate ocellars (short), 3 fronto-orbital (anterior pair convergent, the two posterior pairs reclinate), 1 humeral, 1 presutural, 1-1 notopleural, 1 strong supraalar, 2 postalar, 2 posterior dorsocentral, 1 prescutellar acrostichal, 2 scutellar, 2 sternopleural.

***Pseudiasata nebulosa* Coquillett.**

Male terminalia as figured (figs. 1, 4), the ninth tergite strongly produced ventrad on each side as a "genital forceps," each lobe obviously longer than broad and not tapering, the sides approximately parallel up to the somewhat rounded apex; in anterior aspect, flanking the midline, are two thumb-like processes (anterior claspers?), each of which bears three distinct bristles.

Holotype male: Plummer's Island, Maryland, 1.viii.1902, *H. S. Barber*, taken at light (U.S. National Museum).

One female: Perry, Georgia, 13.iv.1938, *P. W. Fattig* (U.S. National Museum) is probably this species.

***Pseudiasata pseudococcivora*, sp. n.**

Male terminalia as figured (figs. 3, 6), the ninth tergite strongly produced ventrad on each side, the genital forceps so formed being approximately as broad as long, not tapering, and distinctly subtruncate, an appearance especially evident in the anterior aspect; in anterior aspect, the two processes flanking the midline are relatively short and acute, without bristles.

Holotype male, allotype, and eight paratypes: (5 ♂♂, 3 ♀♀), Panama Canal Zone, April, June, and July, 1924 (*D. T. Fullaway*), reared from larvae predacious on

pineapple mealybug; 1 ♂ paratype, "Mexico", intercepted in quarantine at Laredo, Texas, 24.i.1942. Types and paratypes in the U.S. National Museum (Type No. 59179); 2 paratypes (♂, ♀) in the British Museum (Nat. Hist.)

Four puparia with the Canal Zone series curiously resemble the sub-hemispherical, mollusoid puparium of the Syrphid genus *Microdon*, flat below, moderately convex above, oval in outline as seen from above, the spiracular slits on two short, dark red, polished, slightly diverging and slightly swollen processes. The general appearance is like that of *P. brasiliensis*, as figured by Gonçalves (1939).

This is undoubtedly the species which was introduced (as *P. nebulosa*) into Hawaii on several occasions between 1924 and 1932 for the control of the pineapple mealybug (Fullaway, 1933). It may also have been the species reported by Carter (1935) as very abundant throughout Guatemala except in the highlands, feeding voraciously on the same species of mealybug.

One female: Alhajuelo, Panama, 19.iv.1911, August Busck (U.S. National Museum), which was recorded as *Pseudiasata nebulosa* by Sturtevant (1921), is probably *pseudococcivora*, but in the absence of associated males it is not included in the type series.

***Pseudiasata vorax*, sp. n.**

Male terminalia as figured (figs. 2, 5), the ninth tergite rather weakly produced ventrad, each lobe tapering and subconical; in anterior aspect, the two processes flanking the midline are strongly conical, each with two long, dark, conspicuous, widely spaced bristles, and a weak, indistinct subapical bristle.

Holotype male: allotype, and four paratypes (1 ♂, 3 ♀♀), River Estate, Trinidad, British West Indies, January, 1948 (*T. W. Kirkpatrick*), "On *Ps. brevipes* on cacao." Type series returned to the Commonwealth Institute of Entomology, the type to be deposited in the British Museum (Nat. Hist.); 2 paratypes (♂, ♀) in the U.S. National Museum.

Two puparia associated with the specimens, including one adhering to a piece of plant tissue, are of the same curious, *Microdon*-like form as those described under the preceding species.

***Pseudiasata* sp.**

One male: near Moreno's, Pernambuco, Brazil, 4.xii.1931, *M. Kisliuk* and *C. E. Cooley*, "on guava" (U.S. National Museum).

This specimen is very close to *P. vorax* in the form of the male genitalia, especially in lateral aspect, but there is a doubling of the bristles on the anterior processes. A fourth species may be involved, but I shall merely record it here until such time as adequate material is available. The wing pattern is like that of the three preceding species (fig. 7), and not as figured for *P. brasiliensis* Costa Lima.

***Pseudiasata brasiliensis* Costa Lima.**

Costa Lima, 1937, *Chacaras e Quintaes*, **55** (2) pp. 179-182, 6 figs.; Figueiredo, jr., 1938, *Biologico*, **4** (6) pp. 206-207, 4 figs.; Gonçalves, 1939, *Physis*, **17** pp. 103-112, 15 figs.

The male genitalia were not described for *brasiliensis*, and consequently it cannot be compared with the preceding species in that respect. Further, the differences pointed out by Costa Lima in the orbital, tibial, and costal bristles, based only on the descriptions of *nebulosa* by Coquillett and Sturtevant, do not seem to be sufficiently distinct when the holotype of *nebulosa* is compared with the detailed figures given by Costa Lima for *brasiliensis*. With reference to the costal bristles, the description by

Sturtevant is unfortunately misleading, the words proximal and distal being reversed, a *lapsus* which naturally led Costa Lima to use it as one of the significant distinctions between *nebulosa* and his species.

Despite the lack of differences in the above points, however, the pattern of infuscation on the wings appears amply to justify separate specific status for *brasiliensis* (fig. 8). The three photographs of the wings by Costa Lima, and the later drawing by Figueiredo, show clearly that the wings are much more extensively maculated in *brasiliensis* than in the preceding species, and well beyond the range of variation in those species. There are three to five bars or narrow crossbands in the discal cell proximad of the spot which encloses the hind crossvein, a series of three or four

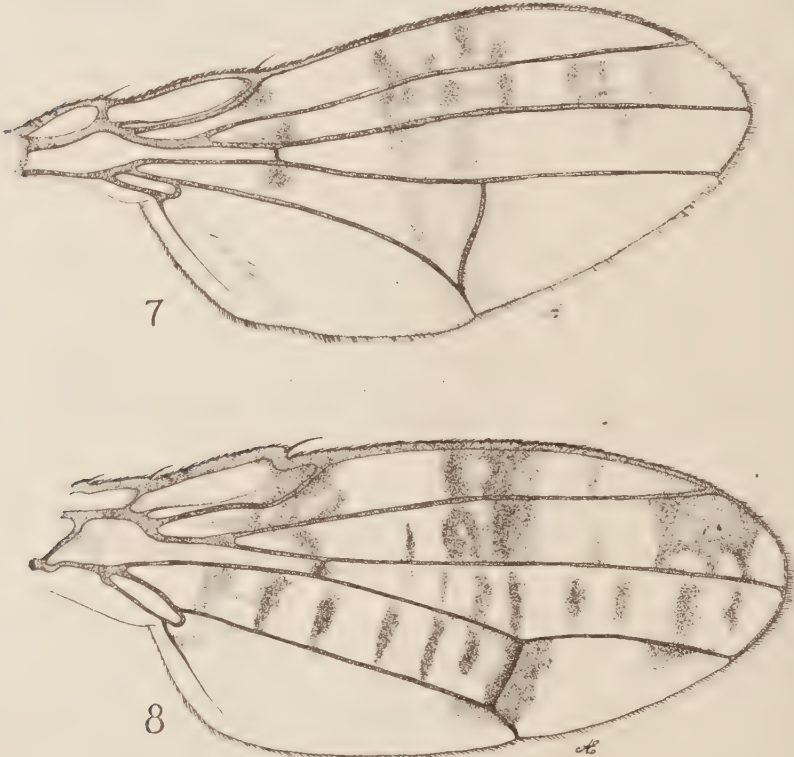


Fig. 7.*—Wing of *P. pseudococcivora*.

Fig. 8.*—Wing of *P. brasiliensis* (♂) (redrawn from Gonçalves).

*By Miss Addie Egbert.

crossbands in the apical cell distad of the level of the hind crossvein, and a narrow crossband in the marginal cell between the broad median and apical bands. In females, the infuscation is more extensive than in the males, and the banded effect is not so pronounced. In all of the preceding species, those areas are predominantly hyaline, with only one roundish spot in the discal cell opposite the small crossvein, and a similar spot in the apical cell as a continuation of the main outer band of infuscation (cf. fig. 7).

Pseudiasata brasiliensis was described from flies reared from larvae predacious on *Pseudococcus brevipes* on the grass, *Eriochloa punctata*, in pineapple plantings in the

state of Rio de Janeiro, Brasil. Figueiredo (1938) has described and figured the larva and puparium, as well as the adult, and Gonçalves (1939) has given particularly detailed descriptions and figures for all stages.

References.

- CARTER, Walter. (1935). J. econ. Ent., **28**, pp. 1037-1041.
COQUILLETT, D. W. (1908). Proc. ent. Soc. Wash., **9**, pp. 144-148.
DA COSTA LIMA, A. (1937). Chacaras e Quint., **55**, pp. 179-182, 6 figs.
FIGUEIREDO jr., E. R. (1938). Biologico, **4**, pp. 206-207, 4 figs.
FULLAWAY, D. T. (1933). Hawaii. For. Agric., **30**, pp. 55-59.
GONÇALVES, C. R. (1939). Physis, **17**, pp. 103-112, 15 figs.
MELANDER, A. L. (1913a). J.N.Y. ent. Soc., **21**, pp. 219-273, 283-300, 1 pl.
MELANDER, A. L. (1913b). Psyche, **20**, pp. 166-169, 1 fig.
STURTEVANT, A. H. (1921). Publ. Carneg. Instn. no. 301. 105 pp., 3 pls., 49 figs.
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THE BIONOMICS AND MORPHOLOGY OF *BRENTHIA LEPTOCOSMA* MEYRICK (LEP., GLYPHIPTERYGIDAE).

By J. R. WILLIAMS, B.Sc., F.R.E.S.,
Department of Agriculture, Mauritius.

Brenthia leptocosma Meyr. was described in 1916 from specimens collected in Mauritius, but it has not been recorded from elsewhere. This Microlepidopteron has an economic interest because it is the only member of the established fauna which inflicts appreciable injury to the plant, *Cordia macrostachya* (Jacq.) Roem. & Schult. (Boraginaceae), a weed which was accidentally introduced, probably from British Guiana, at the end of the 19th century (Wiehe, 1946). *C. macrostachya* is now so abundant that it causes considerable loss to the agricultural community of the Island and an effort is at present being made to control it by biological means (Williams, 1948, 1949).

Distribution and Food-plants.

B. leptocosma is extremely abundant throughout Mauritius except for the comparatively small area of the Island which is above about 1,500 feet, where conditions are unfavourable for the growth of *C. macrostachya*, which is its most favoured food-plant. Williams (1948) states that *B. leptocosma* (= *leptoscoma*) feeds only upon *C. macrostachya*. This is not correct as the moth has since been found to live upon three other species of *Cordia*, *C. myxa* L., *C. abyssinica* K. Sch. and *C. holstii* Gürke. *C. alliodora* Cham., the only remaining representative of the genus in Mauritius, is not attacked.

The restriction of the food-plant range to within the genus *Cordia* queries the endemism of *B. leptocosma* reported by Vinson (1938), for no species of *Cordia* is considered to be indigenous to Mauritius. *C. holstii* and *C. alliodora* were introduced within the last ten years for experimental purposes. The introduction of *C. macrostachya* has already been mentioned; it may be added that its introduction was by means of seed. *C. myxa* is a well known medicinal plant known in most tropical countries as Sapistan, Sebestan Plum, etc. The circumstances of the introduction of *C. holstii* and *C. macrostachya* obviously exclude them as agents whereby the moth could have been introduced. There is, however, no record of the introduction of *C. myxa* and *C. abyssinica*, nor is there any information of *B. leptocosma* before 1916. The possibility of its endemism cannot therefore be completely disregarded, but the improbability of any *Cordia* species being indigenous combined with the apparently complete specificity of *B. leptocosma* to this genus makes the possibility extremely remote.

The great abundance of *B. leptocosma* is due to the excessive growth of *C. macrostachya* in the Island. It is therefore to be concluded that *B. leptocosma* is an example of an insect which has been introduced to a new food-plant which is ideally suited to its economy.

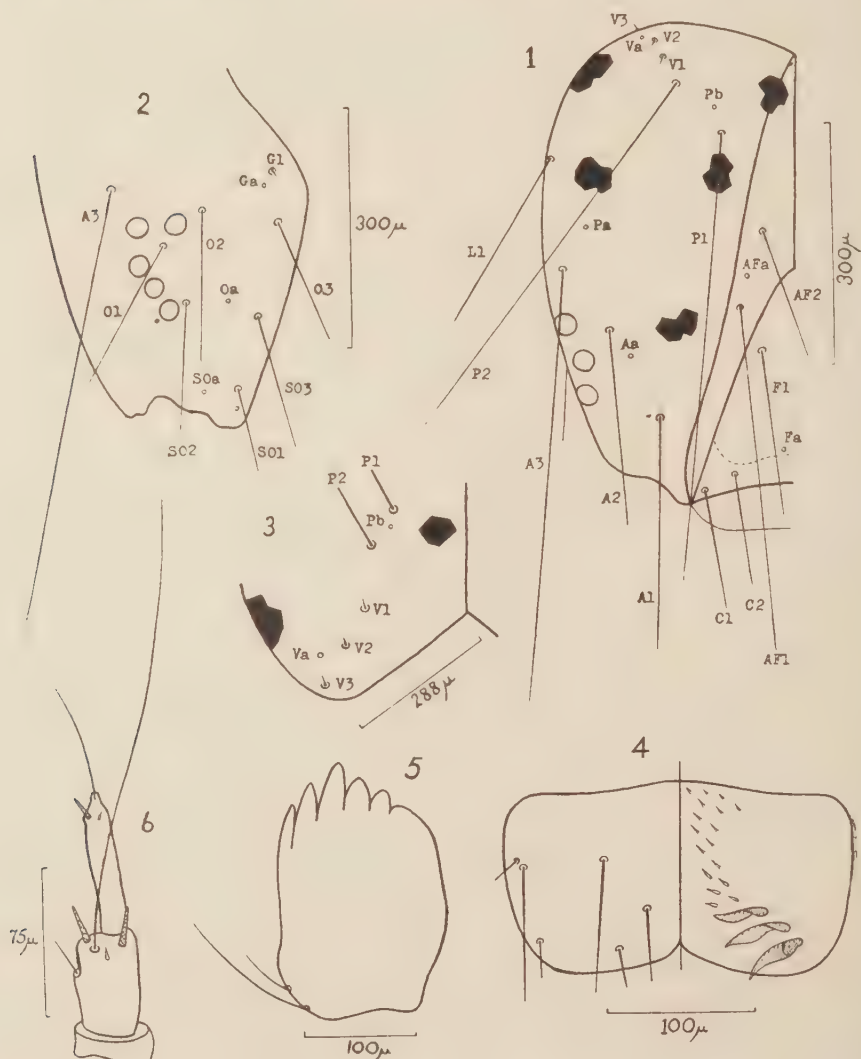
Descriptions of the various Stages.

Egg.—Irregularly round, approximately 0.38 mm. in diameter, flattened, coarsely sculptured, and semi-transparent; assuming a creamy tinge prior to the emergence of the larva.

Mature larva.—As far as the author is aware, larvae of the genus *Brenthia* have not previously been described. No attempt could therefore be made to distinguish

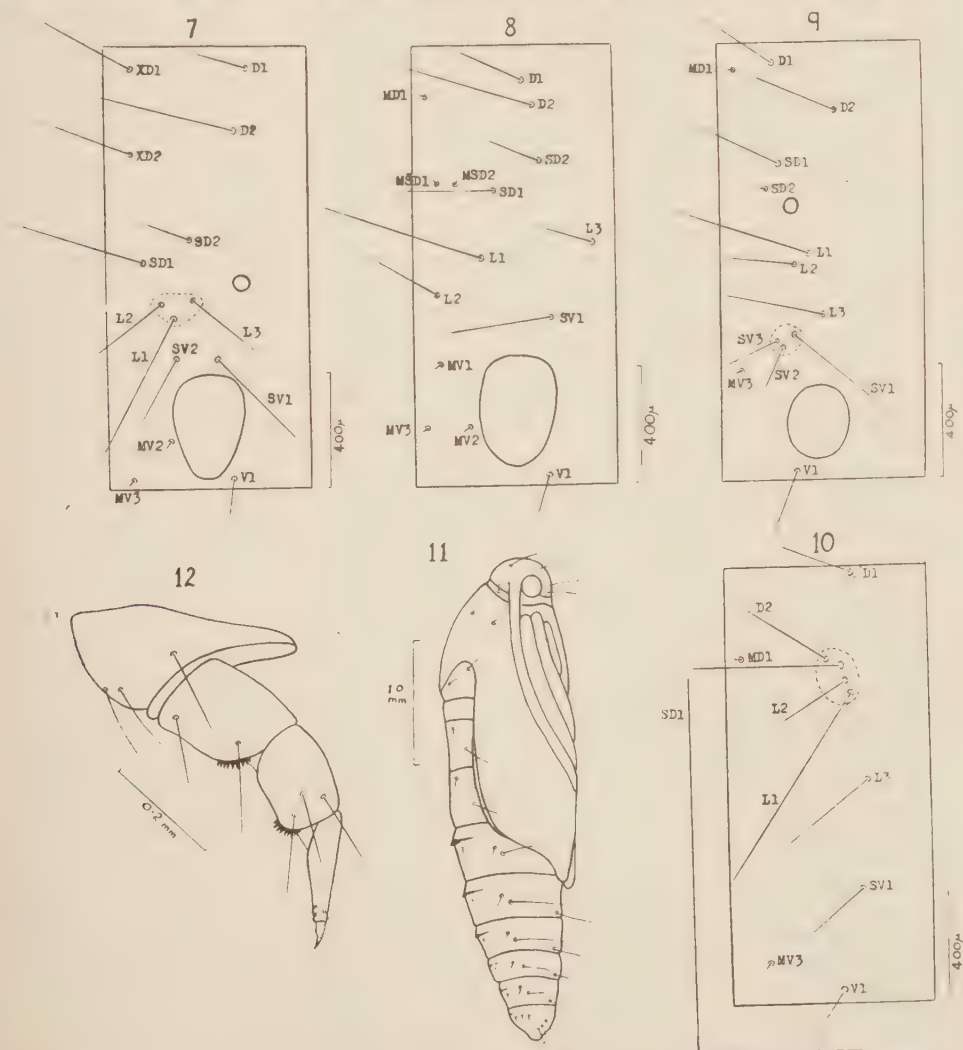
specific from generic characters, the description which follows has accordingly been made in detail. The nomenclature adopted for the setae is that proposed by Hinton (1946).

The mature larva is yellowish-green in general appearance due to the gut contents and the yellow fat body. Pigmentation is confined to the head capsule, the body integument being translucent. *Head* (fig. 1) round, greatest width slightly above middle of face. Integument lightly reticulated. Pigmentation confined to five pairs of regularly positioned black spots, each composed of a number of polygonous areas defined by the reticulations. Vertical triangle obtuse; boundaries of adfrontals joining coronal suture at vertical triangle. Front reaching to middle of face. *Ocelli* (fig. 2) of equal size. Five on each side; space between third, fourth, and



Figs. 1-6.—(1) Head. (2) Side view of head. (3) Arrangement of vertical setae on head. (4) Labrum, epipharyngeal surface on right. (5) Mandible. (6) Antenna.

fifth ocelli less than that between first, second, and third ocelli. *Labrum* (fig. 4) with median incision acute and shallow. Three large flattened epipharyngeal setae; numerous smaller setae and irregularly arranged spines on proximal medial area of epipharyngeal surface. *Mandibles* (fig. 5) symmetrical, with five well developed teeth. *Antennae* (fig. 6) porrect, approximately 0.13 mm. long excluding the setae. Segment I very broad and short; segment III cylindrical, a little longer than segment II and about half its width. *Thorax* with segments patterned dorsally by yellow fat tissue immediately beneath integument. *Legs* (fig. 12) with a comb of short, stout setae along the inner edges of the distal articulations of the femur and tibia. *Tarsus* sub-cylindrical, terminating with a claw. *Abdomen* patterned dorsally by fat tissue as thorax. Segment 5 usually completely yellow dorsally owing to an accumulation of fat. Tapering posteriorly from segment 3. *Prolegs* long, sub-



Figs. 7-12.—(7) Prothorax. (8) Mesothorax. (9) Sixth abdominal segment. (10) Ninth abdominal segment. (11) Pupa. (12) Mesothoracic leg.

cylindrical, length about $2\frac{1}{2}$ times width at base. Crochets uniordinal, arranged in a penellipse. *Chaetotaxy*: Head (fig. 1) with AF1 longer than the clypeal (C1, C2) and frontal (F1) setae, and about $2\frac{1}{2}$ times the length of AF2. Puncture Fa mesad to, and very much anterior to F1, being nearer to C2 than to F1. Seta A3 much longer than A1 and A2. Setae P1 and P2 both very long, about equal in length to A3. Puncture Pa between P2 and A3 and is considerably detached from the posterior group. Puncture Oa (fig. 2) is anterior to all three ocellar setae, and is slightly posterior to a line drawn between SO2 and SO3. Puncture SOa is more than usually anterior to SO2, while there is a puncture near and anterior to SO1. Of the microscopic setae, there is one genal seta (G1); puncture Va is between and lateral to V2 and V3 (fig. 3). Prothorax (fig. 7) with seta MXD1 absent. Mesothorax (fig. 8) with D1 anterior and dorsal from D2; setae SD1 and SD2 are widely separated and form an oblique line, with SD1 antero-ventrad from SD2. This arrangement of the subdorsal setae is identical with that of the prothorax. Seta MV3 is antero-laterad from MV2 and is unusual in being slightly anterior from MV1. On the first eight abdominal segments (fig. 9) seta SD1 is above the spiracle, while SD2 is microscopic and antero-dorsad from the spiracle. Of the lateral group L1 is the longest, it is near and postero-dorsad from L2. Seta L3 is some distance postero-ventrad from L2. The three subventral setae are on a common tubercle. Seta SV3 is, like SV2, antero-ventrad from SV1, with SV2 more ventrad than SV3. The latter is slightly longer than SV2. Seta MD1 is opposite D1. The homotypy of the setae of the ninth abdominal segment is exceedingly difficult to resolve. Four setae are on a common tubercle (fig. 10), these are apparently D2, SD1, L1, and L2. Setae D1 are on the same tubercle and are dorsal and *behind* the setae D2. Seta SD2 is absent, while SD1 is very long—the longest seta on the body. Seta MD1 is opposite D2.

Pupa.—Approximately four mm. long. Pale yellow, darkening with age. Colour of eyes changes similarly from pale pink to black. Numerous setae are present (fig. 11) while on segments 5, 6, 7, 8, 9, and 10, there is a dorsal row of small, strong, caudally reflexed spines. Either three or four abdominal segments are movable, these are 5, 6, 7, and 5, 6, 7, 8, respectively.

Adult.—This was described by Meyrick (1916), who refers to it as being "very like *cyanaula* in markings, but palpi quite different and characteristic".

Life-history.

The adults are most active on hot sunny days when they are to be seen in large numbers hovering around *Cordia* bushes. In the hot season (Dec.–April) they are often found in swarms of many hundreds flitting amongst the foliage.

The eggs are laid singly on the under surface of a leaf along the sides of the larger veins, in the angle formed by the vein and the leaf blade. The incubation period is 9–10 days and larval movements become visible through the chorion about two days before hatching.

The newly hatched larva is about 1 mm. long and is creamy translucent. Feeding commences almost immediately, the ingested leaf tissue giving the larva a green hue; it is confined to the under surface of the leaf throughout development, small circular patches being consumed, leaving the upper epidermis intact. The leaf area over which the larva is active is enclosed by a finely woven web in which are incorporated the faecal pellets and the unpalatable urticating hairs of the leaf. As development proceeds, the circles of destroyed leaf tissue become confluent, resulting in an irregular area consisting only of the upper epidermis and the veins. The limits of the area so skeletonised follow the larger veins.

At one point in the leaf area occupied by the larva is a circular hole (fig. 13). This is of just sufficient diameter to allow the larva to pass through to the upper

surface of the leaf. The function of this hole is apparently that of an escape passage. When at rest the larva faces the hole directly, and when touched or pricked, darts through it on to the upper surface of the leaf, leaving only its hind prolegs visible from the under surface. The hole is therefore to be regarded as a means of avoiding parasitism. The larva returns to the under surface of the leaf within a few seconds by wriggling backwards. If it is forced to leave the immediate vicinity of the hole while on the upper surface, it fails to return. The "escape" hole is usually situated in the angle formed by two veins.



Fig. 13.—Diagrammatic view of the under surface of an attacked leaf with the larval webbing omitted: (a) "escape hole"; (b) position of larva when at rest; (c) skeletonised area.

Under insectarium conditions, where the life-cycle was worked out at a mean temperature of 24°C. and a relative humidity of approximately 76 per cent., there were five larval instars. The width of the head capsule was measured with 25 larvae of each instar, and the mean width and Standard Deviation calculated. The results are given in Table I. The duration of larval life was 27–29 days.

TABLE I.

	Mean head width (mm.)	S.D.	Approx. length (mm.)
1st instar	0.171	0.004	1.3
2nd "	0.241	0.004	—
3rd "	0.340	0.018	3.4
4th "	0.473	0.066	—
5th "	0.670	0.053	7.0

There is a prepupal stage of two days when feeding ceases and the larva becomes a uniform grey colour. The cocoon is spun under the larval webbing and consists of three envelopments of very flexible but tough webbing. Its overall length is about 1 cm.

The pupal stage lasts for 12-13 days. The mobile segments of the abdomen give the pupa considerable power of locomotion. The fused terminal segments are used as a lever, and with the assistance of the dorsal rows of spines the pupa is able to force its way through the looser fibres at one end of the cocoon, thus enabling the adult to emerge. The last few abdominal segments are not withdrawn from the cocoon.

Extent of Damage.

An unsuccessful attempt was made to correlate the percentage of leaf attack with the number of flowers, fruits, and seeds. Some of the data collected serves, however, to illustrate the large number of larvae, and the incidence of leaf injury, which is evident on bushes of *C. macrostachya* throughout the Island.

In one locality 60 bushes were chosen at random. The number of leaves, attacked leaves, larvae, and pupae per plant were counted. The results are summarised in Table II.

TABLE II.

No. of plants	Mean No. of leaves	Mean Percentage leaf attack	Mean No. of larvae	Mean No. of pupae
60	358	41	40	2

The maximum percentage of leaf attack was 82 per cent. on a bush with 721 leaves, the minimum 12 per cent. on a bush with 132 leaves. The maximum number of larvae counted on a bush was 135, and the minimum 2. The figures tend to exaggerate the actual damage caused by *B. leptocosma* as it must be borne in mind that a larva confines itself to one leaf and destroys only a portion of it. The large difference between the number of attacked leaves and the number of larvae on a bush is evidence that an attacked leaf is not usually sufficiently mutilated to wither and fall. Also, leaf injury is more severe on plants with succulent foliage; such plants are found in moist, shaded habitats where leaf growth is more vigorous and damage consequently of less detriment to the plants. Young leaves are rarely attacked, there being a decided preference for the larger, older leaves which are usually shaded from the direct rays of the sun by the newer growth.

Parasites.

The following parasites, identified by the Commonwealth Institute of Entomology, are recorded for the first time from *B. leptocosma*.

Dimmockia nigra (Masi) (Eulophid).—A solitary external parasite of the pupae. The fully developed parasite larva pupates within the host cocoon. The pupa has the heavy integument and jet black colouration typical of the sub-family EULOPHINAE. The adult emerges by biting a small hole through the side of the host cocoon. Numerous specimens obtained.

Microgaster curticornis Granger (Braconid).—A larval parasite, the cocoon of which is found under the larval webbing of *Brenthia*. Four specimens obtained.

Chelonus (*Chelonella*) sp. probably new. (Braconid).—A solitary internal parasite of the larvae which emerges when the cocoon is completed, and within which its own cocoon is spun. Numerous specimens obtained.

Mesochorus sp. nr. *nigellus* Wilkinson (Ichneumonid).—A secondary parasite, probably hyperparasitic upon *Chelonus*, for it emerges from a cocoon within that of *Brenthia*. Three specimens obtained.

Genus near *Spinolia* sp. (Ichneumonid).—A secondary parasite, possibly a hyperparasite of *Microgaster*. Two specimens only were obtained: these emerged from parasite cocoons under the larval webbing of *Brenthia*.

Calliceras fipensis Ferrière (Calliceratid). A secondary parasite. Six specimens were reared from a cocoon which was probably that of *Microgaster*.

Tetrastichus sp. (Eulophid).—Host affinities uncertain. Nine specimens were reared from a spherical cocoon under the larval webbing of *Brenthia*.

In addition to those listed, one specimen of an unidentified Chalcidoid and an Ichneumon have been found. The former is a pupal parasite, and the latter a (secondary?) larval parasite.

Summary.

Brenthia leptocosma is a Microlepidopteron which feeds upon the Boraginaceous bush, *Cordia macrostachya*. This plant, since its accidental introduction into Mauritius about 1900, has become a pest of considerable agricultural importance. Other food-plants are *C. myxa*, *C. abyssinica* and *C. holsti*, which are all unimportant trees. *B. leptocosma* has been recorded only from Mauritius. It is not likely to be indigenous for it is restricted to *Cordia* species, none of which are considered to be native to Mauritius.

The morphology and biology of the various stages are described.

The distribution of *B. leptocosma* is that of its food-plants: it is extremely numerous in all regions below about 1,500 feet, which is the upper limit for *C. macrostachya*. The abundance of the moth is due to the excessive growth of *C. macrostachya*. Owing to its mode of life, it is of no value as a controlling agent of *C. macrostachya*.

Larval and pupal parasites are recorded.

Acknowledgement.

The author is indebted to Mr. F. Nadeau and Mr. P. R. Hermelin for their able assistance.

References.

- HINTON, H. E. (1946). On the homology and nomenclature of the setae of lepidopterous larvae, with some notes on the phylogeny of the Lepidoptera.—Trans. R. ent. Soc. Lond., **97**, pp. 1-37, 24 figs.
- MEYRICK, E. (1916). Exotic Microlepidoptera, **1**, p. 560.
- VINSON, J. (1938). Catalogue of the Lepidoptera of the Mascarene Islands.—Mauritius Inst. Bull., **1**, pt. 4, pp. 1-69.
- WIEHE, P. O. (1946). The control of *Cordia macrostachya* (Jacq.) Roem. & Schult.—Publ. Mauritius Govt. no. 28, pp. 11-43, 3 pls., 2 maps, 8 figs.
- WILLIAMS, J. R. (1948). A preliminary account of the project for the control of *Cordia macrostachya* (Jacq.) R. & S., in Mauritius.—Rev. agric. Maurice, **27**, pp. 214-233.
- WILLIAMS, J. R. (1949). Rep. Dep. Agric. Mauritius, 1948, pp. 63-69.

ON THE IDENTITY OF AN ICHNEUMONID PARASITE OF *HEPIALUS LUPULINUS* (L.).

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A paper by the present writer on an Ichneumonid parasite of *Hepialus lupulinus* (L.) under the name *Alomya debellator* (F.) appeared in the last issue of this journal (1950). It now transpires that this Ichneumonid is not *A. debellator* but *Ichneumon suspiciosus* Wesm. This error was pointed out, as soon as the paper was published, by Mr. J. F. Perkins of the British Museum, and agreed by Mr. G. J. Kerrich of the Commonwealth Institute of Entomology, both of whom, as specialists in the ICHNEUMONIDAE, recognised the illustration produced from a drawing prepared by Mr. A. J. E. Terzi as representing a species of *Ichneumon*, probably *I. suspiciosus*. The actual specimen illustrated has been traced, and its identification has been confirmed by Mr. Perkins as *Ichneumon suspiciosus* Wesm. It is certain, moreover, that all the parasite material involved was this species and not *A. debellator*.

The mistake arose through the taking over, by the writer, of an investigation of certain Hepialids and their parasites begun by the late Mr. S. Garthside, whose tragic death occurred towards the end of 1939. Garthside's preliminary notes and specimens were handed on as referring to *Alomya debellator*, and there was then no reason to doubt that he had submitted specimens to the Commonwealth (then Imperial) Institute of Entomology for identification, in accordance with his usual custom. A thorough search through the Institute's files confirms, however, that he did not do so.

It is evident that Garthside was misled into believing that the parasite with which he was dealing was *A. debellator* by a paper by Waterston (1926) in which Stenton is quoted as having discovered females of *Alomya* in the tunnels of Hepialid larvae, and as having observed the adult parasites pairing and ovipositing. Stenton's observations, however, were not elaborated; he did not bring his experiments to completion, and his evidence must be regarded as inconclusive.

The Hepialid investigation was again disrupted through circumstances arising out of the war, and it was never completed to the writer's satisfaction.

Now that the identity of the parasite has been established, it should be noted that the systematic section of the paper under reference does not apply, and that the data on the developmental stages should be taken as referring to *Ichneumon suspiciosus*.

Further, many ICHNEUMONIDAE pass the winter as mated females, and *Ichneumon suspiciosus* has actually been recorded by Hancock (1925) as hibernating both under bark and in tufts of the grass, *Deschampsia caespitosa*. This accounts for the finding of its primary larvae in the larvae of *H. lupulinus* at various times in the spring.

The co-operation of Mr. Kerrich and Mr. Perkins in the preparation of this note is gratefully acknowledged.

References.

- CAMERON, E. (1950). The biology and economic importance of *Alomya debellator* (F.), a remarkable parasite of the swift moth *Hepialus lupulinus* (L.). Bull. ent. Res., **41**, pp. 429-438.
- HANCOCK, G. L. R. (1925). In Gardiner, J. S. The Natural History of Wicken Fen. A preliminary account of the Ichneumonidae of the Cambridgeshire Fens, **2**, pp. 122-139.
- WATERSTON, J. (1926). A note on *Alomyia debellator* Fab.—Ent. mon. Mag. **62**, pp. 98-99.

STUDIES ON THE OX WARBLE FLIES, *HYPODERMA LINEATUM* AND *HYPODERMA BOVIS*.

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These studies were undertaken with a view to elucidating certain aspects on the bionomics of the two species of Ox Warble Fly, *Hypoderma lineatum* (Vill.) and *H. bovis* (de Geer) and to determine the value of dichloro-diphenyl-trichlorethane (DDT) and benzene hexachloride (BHC) for their control. The studies were conducted in South Wales over several years, culminating in intensive investigations in 1945 and 1946. The delay in the publication of the results is largely due to the departure of one of the writers shortly afterwards to take up his present appointment in the University College of the Gold Coast.

Historical.

The earliest references to ox warbles are found in the literature of Roman times where the phenomena of gadding of cattle in summer and of swellings on their backs were recorded. Although these records cannot be considered of much scientific significance, they do indicate the existence of the ox warble flies as pests of cattle in this early period. The gadding was associated with Tabanid flies by these early observers but the actual parasite responsible for the warbles was unknown to them. Warburton (1922) states that the first records of scientific value were made by Vallisnieri (1733) who spent much time and money studying warbles and who ultimately succeeded in breeding out a single, but rather damaged, specimen of the adult fly which he described.

According to MacDougall (1934), Linnaeus (1739) was also interested in warbles but unfortunately he confused the Ox Warble Fly, *Hypoderma* sp., with the Horse Bot Fly, *Gastrophilus equi* (F.). Réaumur (1738), on the other hand, was more fortunate even though he found his neighbourhood around Paris to be free from warbles. He obtained two affected heifers from a wooded locality in the vicinity of Brie l'Abbaye where cattle were known to be badly infested. By keeping these animals under close supervision, he was successful in observing the emergence of the larvae from the swellings or tumours on their backs and in breeding out the flies in due course.

The first record of ox warble fly having been caught in the open is by de Geer (1776) who captured a "bee-like fly" in the field which he later recognised as identical with Réaumur's specimens and named *Oestrus bovis*. De Villers (1789) described an insect troubling cattle under the name of *Oestrus lineatus* but its bionomics remained unknown until much later. The genus *Hypoderma* was established by Latreille (1825) and the two species became known as *H. bovis* (de G.), and *H. lineatum* (Vill.).

Clark published a series of works on ox warble flies in 1797, 1815, 1827 and 1843 and gave details of a method, involving the use of a small sleeve, for collecting the full-grown larvae on their departure from the back of the animal to pupate. Essentially, the method consisted of fixing a small muslin bag over each tumour but even with this technique the difficulty of breeding out the adults remained a serious problem. This is reflected in the small number of specimens used by Joly (1846) and Brauer (1863), each basing his anatomical studies on about three specimens. Various hypotheses were advanced to explain the life-history, method of oviposition and the mode of entry of the larvae into the body of the host. Clark (1815) suggested that the ovipositor of the ox warble fly constituted a piercing organ. Ormerod (1885-95) accepted this supposition at first but later decided to abandon it. She then presumed that the eggs were deposited on the back of the animal and advised smearing this region with oil in the summer to deter oviposition. Hinrichsen (1888) maintained that the newly hatched larvae were picked up with the grass and that these bored their way into the oesophageal wall where they spent five months prior to proceeding by way of the spinal canal to their ultimate position in the back of the animal.

The exact method of oviposition was first described by Riley (1892) and it was confirmed by Carpenter & Steen (1908) for *H. bovis* and by Hadwen (1915a) for *H. lineatum*. Carpenter (1914) conducted experiments to test the validity of the hypothesis that cattle in licking themselves transfer the eggs to their digestive tract. By muzzling the experimental cattle to restrain them from licking themselves during the oviposition period of the flies, he found no decrease in the number of warbles in the backs of the cattle and concluded that the eggs were not transferred in this manner. Hadwen (1915b) succeeded in establishing the fact that first stage larvae soon after hatching from the eggs are capable of penetrating the skin of cattle, by watching their activity under a microscope, on a piece of skin freshly removed from a cow. Similar observations were also made by Carpenter (1914) and gradually the bionomics of the ox warble flies became better known.

Later workers concentrated mainly on the control of the two species and researches to this end have been in progress for the past quarter of a century. The first major attempt to evolve a satisfactory method of control in Britain was begun in 1918 when a Departmental Committee was appointed by the Government to conduct observations and experiments. A Committee was appointed by the Leathersellers' Companies in 1929, for the same purpose. In these national campaigns, a wash consisting of derris root, soft soap and water proved a most successful dressing. In 1936, the Warble Fly (Dressing of Cattle) Order was issued by the Ministry of Agriculture & Fisheries compelling all cattle owners to dress their animals with this mixture but in 1940 it was suspended on account of the stringencies of war-time conditions.

Economic Importance.

The adult flies, in common with certain species of Tabanids, cause cattle to gad, the udders being bruised by striking against the body and legs and resulting in an appreciable reduction in milk yield. Observations made by the writers indicate that the milk yields are often reduced by about 10 per cent. on days when gadding is prevalent. In addition, excitement and over-exertion frequently cause cows in-calf to abort, with the result that not only the calf is lost but also the milk yield for the whole lactation period is seriously diminished, often to one half of the normal production.

No doubt much irritation and pain is suffered by the animals while the larvae burrow through the skin and later during their extensive wanderings through the different parts of the body. The tissues in the sub-cutaneous regions of the back are frequently inflamed and when such animals are slaughtered the flesh, which is at first straw-coloured and jelly-like in appearance, turns into a dirty green, congealed mass, unfit for human consumption within a few hours.

Bacteria sometimes enter through the breathing holes made by the larvae in the skin and result in extensive abscess formation and in serious loss in body condition of the animal. While animals are attempting to avoid the flies by standing in water or by some other means, they lose valuable feeding time essential for maintenance of high milk or beef production. The greatest obvious financial loss is reflected in the depreciation of the affected hides. The perforations occur in the middle region of the hide, rendering it useless for the most important purposes in commerce.

Methods.

The present studies were made principally in Glamorgan, South Wales, and the parishes of Pencoed, Llanilid and St. Mary Hill within this county became the chief localities for the most intensive observations on account of their varying topography and accessibility to the University College of Wales, Cardiff. For the detailed data on warble infestations, weekly counts of the number of larvae, indicated by the characteristic warble-swells with the breathing pores on the backs of the cattle, were made both in 1945 and 1946 from January to August on many farms. Information on the exact time of the year when the larvae of each species appear in the sub-cutaneous tissues of the backs of cattle was gathered by examining the hides of the cattle killed at the abattoir in the vicinity of Pencoed, Glamorgan, and removing all the warbles for identification in the laboratory. The abattoir was visited weekly from the beginning of February to about the end of July, 1945, when a minimum of 24 flayed hides were examined for the presence of larvae on each occasion. As the animals slaughtered at this centre came from all parts of Glamorgan, it can be claimed that the information obtained was representative for the entire county. Most of the warbles removed from the hides were either in their fourth or fifth larval stages, the remainder being in the third stage. The larvae in the third stage proved rather more difficult to identify than those in the later stages of growth. The main distinguishing characteristics used in the present investigations were as follows :—

(a) *Third stage or antepenultimate larvae.* It was found necessary to clear, mount and examine all specimens under high power magnification and depend on the mouth hooks and cephalo-pharyngeal skeleton for separating the larvae of the two species. The third stage larvae moult soon after reaching the back of the animal and cast off the skeletal parts, including the mouth parts, and it therefore becomes necessary to rely on different features for distinguishing the larvae of the later stages.

H. bovis

1. Mouth hooks with anterior ends forked at the tips; posterior ends blunt.
2. Spine between the mouth hooks pointed anteriorly, but somewhat blunt posteriorly.

H. lineatum

1. Mouth hooks with anterior ends not forked; both anterior and posterior ends pointed.
2. The spine between the mouth hooks pointed both anteriorly and posteriorly.

(b) *Fourth stage or penultimate larvae.* The specific characters can be recognised under low magnification when the larvae are placed vertically with their posterior stigmal plates upwards.

H. bovis

1. Stigmatal plate with 26 to 38 discs but normally about 30.
2. The discs connected or fused together, giving the plate a darker appearance than that in *H. lineatum*.

(c) *Fifth stage or ultimate larvae.* Although characteristic differences between the larvae of the two species, at this stage of growth, can usually be detected with the unaided eye, it was found advisable to examine them under low power magnification for diagnosis with any degree of accuracy.

H. bovis

1. Surface of the stigmatal plate cup or funnel shaped.
2. Spiny armature absent on the ventral surface of the last two segments.
3. Light coloured region between the incurving borders around the tracheal opening of the respiratory area small and narrow.

H. lineatum

1. Stigmatal plate with 14 to 28 discs but normally about 20.
2. The discs loosely bound or in groups giving the plate a lighter appearance than that in *H. bovis*.

H. lineatum

1. Surface of the stigmatal plate flat.
2. Spiny armature absent only on the last segment.
3. The corresponding area larger and wider, being about twice as extensive as that in *H. bovis*.

Behaviour of Adults.

As the adult of both species are exceedingly rapid in flight, critical observations on their behaviour in the field is extremely difficult. No significant difference was observed in the seasonal activity of the adults in South Wales from that in most other parts of Britain. The adults of *H. lineatum* started to emerge from the puparia in 1945 and 1946, under outdoor conditions, early in May and those of *H. bovis* about four weeks later. The adults were seen on the wing in the open from May until the beginning of September with maximum activity in the latter part of June and in July. They dislike rain as well as high winds and become active only in hot and sunny or sultry weather. This was well exemplified on two farms in 1945 when adults were detected on the wing on May 10th and 11th, the first indication of their presence on each occasion being their characteristic "hum" and the fact that cattle, which had been grazing peacefully, began gadding immediately. The weather on May 10th was hot and sunny and on May 11th warm and sultry. The cattle on both farms had access to comparatively high and exposed ground where they congregated soon after being disturbed by the ox warble flies. It was frequently noticed in the case of open common land of varying altitudes, that whenever the flies were persistent, the cattle neglected the lower regions with their better pastures for the higher ground and poorer swards, although it often involved travelling at least a mile. Even on the same farm, cattle on the low sheltered pastures would be gadding madly in contrast to those on the more exposed fields.

The adults of both species avoid the vicinity of water and shade and it was observed on numerous occasions in the course of these studies that whenever the flies became active the cattle immediately sought, if available, rivers, ponds, open sheds or shade provided by trees or buildings, and stood there quite peacefully for hours. Claims have been made from time to time that gadding should not be associated with ox warble flies as, in contrast with the troublesome species of Tabanids, they do not possess mouth parts capable of causing any injury or pain to cattle. It was found by the writers that such claims are not justified as cattle are unable to differentiate between the humming noises of the adults of *H. bovis* and *H. lineatum* and those of the larger species of Tabanids. It is noteworthy in this connection that one of the writers by creating a hum somewhat reminiscent of that made by these two types of flies when in flight could cause gadding among cattle at least on hot, sunny days during the summer months.

As expected, it was discovered that cattle constituted the principal host but horses were found slightly infested in some localities. The most seriously affected horses were observed at riding schools in the vicinity of Cardiff. The lesions caused by the larvae directly beneath the saddles often developed into painful sores which incapacitated the animals for any useful purpose for some time.

Positions occupied by the Larvae in the vertebral Region of their Host.

The majority of the warbles in the fourth and fifth stage of their development occur in two bands, about seven to eight inches in width, on either side of the vertebral column. The two bands are separated from one another by about two inches and extend from the shoulders to the hip bones. The narrow strip between these two bands and directly dorsal to the spinal cord normally remains free of infestations. A few warbles are found occasionally in the tissues overlying the proximal half of the ribs and the dorsal region of the shoulders as well as in the areas immediately posterior to the hip bones (tuber sacrale), on the part dorsal to the sacrum and at the base of the tail. The larvae of both *H. lineatum* and *H. bovis* occur in all these different locations.

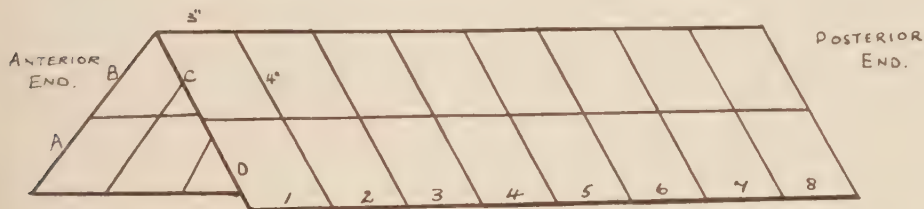


Fig. 1.—Device for establishing exact location of larvae of *H. lineatum* and *H. bovis* in the vertebral region of the host.

Length of Time spent by the Larva in the vertebral Region of its Host.

In order to obtain information on the length of time spent by the larvae in the vertebral region, it was found necessary to establish the exact location of each individual larva on its arrival in this area. For this purpose, a special device was used which consisted of a stout, wire frame, measuring 24 inches long and 16 inches wide. This frame was converted into a network of 32 interstices, each 3 x 4 inches, by fixing to it three strands of thin wire lengthwise and seven strands in the opposite direction (fig. 1). When in use the device was orientated in such a way on the back of the animal that it extended from the hip bones almost to the shoulders with 16 interstices on each side of the vertebral column. On all occasions when counts were made, great care was exercised to ensure that the apparatus always occupied the same position. The individual rows of interstices running lengthwise were indexed A, B, C and D and numbered 1—8 within each row. This method was found convenient for plotting on a specific card for every animal the exact position occupied by each warble on its vertebral region. On these cards, records were also kept of the dates on which all the larvae arrived at their respective locations and left for pupation.

These investigations were undertaken at three farms and involved 78 cattle, 7 of which remained free from infestation throughout the season. The average length of time spent by the larvae in the back of their host on the different farms is given in Table 1.

TABLE I.

Average length of time spent by Larvae in the vertebral Region of the Host.

Farm	No. of Larvae involved	Average No. of Days
1	153	44.24
2	144	41.12
3	207	42.14
Total = 504		Mean = 42.50

Essentially, these findings confirm those recorded by previous workers. Blagoveschenskii (1930) found that the average period spent by warbles in the backs of cattle in Russia is approximately 45 days, while Gaut (1930) has indicated that the minimum time in Britain, at least in the Midlands, lasts not less than 38 days. It must be emphasised that it is extremely difficult to state with any degree of certainty the minimum period, due to the small size of the larvae when they arrive in the subdermal tissues.

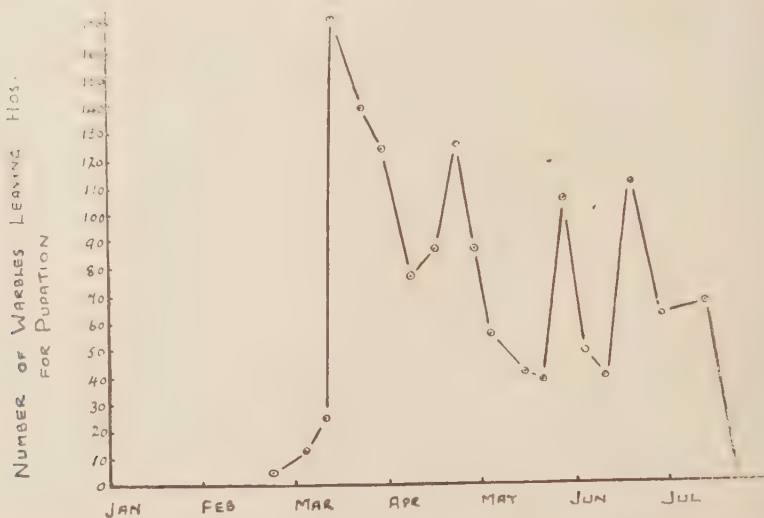


Fig. 2.—Seasonal curve indicating when larvae of *H. lineatum* and *H. bovis* left the host for pupation in 1945.

The Period when most Larvae leave the Host for Pupation.

In 1945, weekly counts of the warbles leaving the vertebral region to pupate were made on 171 cattle at Pencoed, Glamorgan from early February until the end of July. The results are recorded graphically in Fig. 2. It was found that the larvae first began to leave the cattle during the last week in February. By the end of February, approximately 1 per cent. of the total number of warbles found on all the cattle during the entire season had emerged. Thereafter, the numbers of larvae emerging increased rapidly and attained a peak in the third week in March. About 32 per cent. of the total population of larvae for the whole season reached maturity and left the host in March, 22 per cent. in the second and third week of this month. A gradual decrease in the number leaving followed until mid-April when a slight

increase took place for about a week. After this period, there was a continuous reduction throughout the fourth week of April and the first three weeks of May. Two further peaks of larval emergence occurred, one at the end of May and the other in the third week in June, but both peaks were less pronounced than that in April. During April, 26.2 per cent. of the total population of larvae for the year left to pupate compared with 32 per cent. in March, 17.5 per cent in May, 18.4 per cent in June and 4.9 per cent in July.

In 1946, weekly counts of the larvae which had become fully grown were made on 125 cattle at the same centres as in 1945. The results are presented graphically in fig. 3. The larvae commenced to leave the cattle as early as the second week of February and by the end of this month 2.5 per cent. of the total population of the larvae recorded during the entire season had emerged. There was an increase in the number of warbles leaving during early March, rising to a peak in mid-March. This was followed by a temporary decline in the third week, but subsequently there was a rapid rise to the highest peak of the year early in April. About 28 per cent. of the larvae attained full-growth in March and 32 per cent. in April. From mid-April to the third week of May, the rate at which the larvae were reaching maturity and leaving the cattle showed a decrease which was followed later in the season by a further peak in the third week in May and a rapid decline in early June. Subsequently, the number of warbles leaving the vertebral regions of the animals increased and attained another peak at the beginning of July, and then dropped rapidly. The rate at which emergence took place in May, June and July, based on the total number of larvae recorded on the cattle for the whole season, was 26.4, 9.0 and 1.5 per cent., respectively. A comparison of the rate at which the larvae attained maturity and emerged from the cattle to pupate in the different months of 1945 and 1946 is given in Table II.

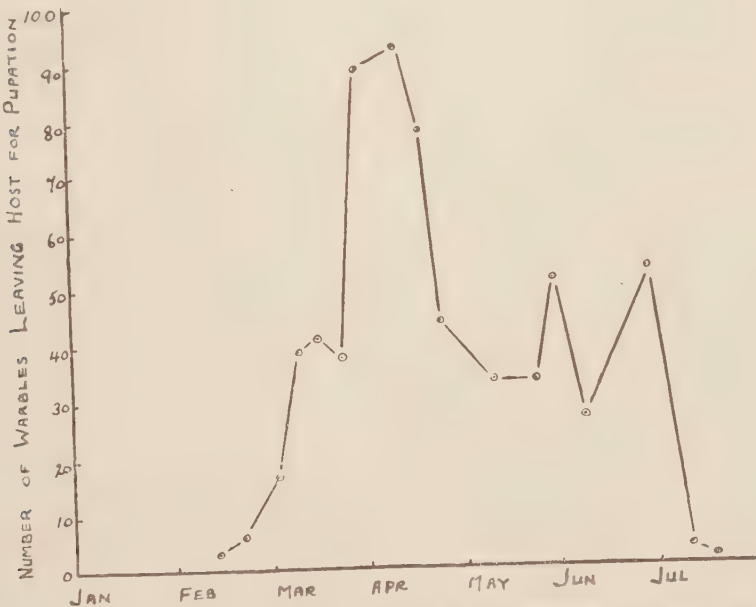


Fig. 3.—Seasonal curve indicating when larvae of *H. lineatum* and *H. bovis* left the host for pupation in 1946.

TABLE II.

Percentage Rate at which Larvae reached Maturity
and left to pupate.

Month	1945	1946
February ..	1.0	2.5
March ..	32.0	28.0
April ..	26.2	32.6
May ..	17.5	26.4
June ..	18.4	9.0
July ..	4.9	1.5

It is evident from Table II and figs. 2 and 3 that the rate at which the larvae were leaving the host followed the same trend in 1945 and 1946, except that it was much more rapid in the latter season, 89.5 per cent having left by the end of May compared with 76.7 per cent. in the former year. This earlier pupation, in the case of 12.8 per cent. of the total infestation of larvae for the whole season, in 1946 when compared with the data for 1945 may have been due to a difference in the climatic conditions during the oviposition period of the flies in 1944 and 1945 but an examination of the meteorological records for the area in these two seasons yielded no definite evidence.

Apart from the biological interest, information on the period when the maximum number of larvae leave the host to pupate has a fundamental bearing on the correct timing of application of insecticides for the control of *H. bovis* and *H. lineatum*. The only practical means of controlling these insects under commercial conditions hitherto discovered include the application of a derris-root wash to the backs of affected cattle in such a way that it destroys the warbles whilst still *in situ* within the subcutaneous tissues. A minimum of four applications are imperative. Gaut (1930) recommended the following periods for dressing the cattle in Britain with derris insecticides :—

First application	..	17th–19th March.
Second application	..	14th–16th April.
Third application	..	12th–14th May.
Fourth application	..	16th–18th June.

Gaut (1930) claimed that the percentage of larvae pupating before 28th March is as low as 1.4 per cent. It was found in the present studies, however, that in the county of Glamorgan, in 1945 and 1946, over 20 per cent. had dropped off the cattle before this date. It would seem, therefore, that the "warble season" is somewhat earlier in South Wales than that normally assumed for Britain and based largely on experience gained in Worcestershire. The results of the present investigations strongly suggest that in order to obtain the maximum destruction of warbles on cattle, at least in South Wales and possibly many other areas in Britain, the insecticides should be applied as follows :—

First application	..	8th–10th March.
Second application	..	7th–9th April.
Third application	..	9th–11th May.
Fourth application	..	12th–14th June.

In view of the comparatively high populations of larvae sometimes present on cattle in July in localities where *H. bovis* is the dominant species, it would be desirable to make a fifth application of the insecticide in the second week in July in such districts.

Seasonal Incidence of the Larvae in the vertebral Region of their Host.

The general observations made by one of the writers over a number of years in the Midlands, and more recently by both writers in South Wales and Monmouthshire, on the incidence of warbles in the subcutaneous tissues of the backs of cattle, revealed striking differences in the seasonal appearance of the larvae. The results of the counts of larvae present that were made each week from the beginning of January to the end of July on a number of farms in Glamorgan in 1945 and 1946 also showed marked variations from one season to another and particularly between different localities within the same year. The four main types of typical variations encountered in these studies in the county of Glamorgan are presented graphically in figs. 4, 5, 6 and 7.

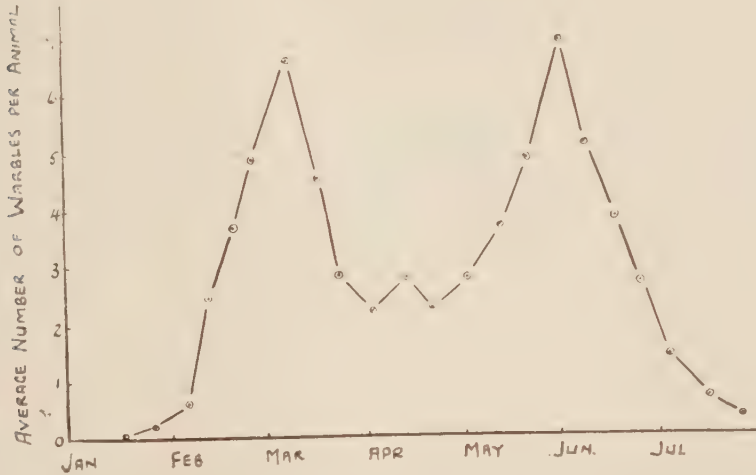


Fig. 4.—Type A. Seasonal incidence of larvae of *H. lineatum* and *H. bovis* in the vertebral region of the host.

(a) Types of Variation.

Type A. In this type of seasonal incidence (fig. 4), the larvae began to appear in subcutaneous regions of the back in the latter half of January, though an occasional specimen was noted as early as December in 1944 and 1945. Such an occurrence is much earlier than any previously recorded in Wales. According to Walton (1925), the second week in February is the earliest time of the appearance of warbles on cattle in North Wales but Davies (1930) observed them in the same area as early as the second week in January. In Type A, the number of larvae arriving in the back increased rapidly throughout February to reach the first high peak in the early part of March. The larval population then decreased just as quickly until the first week in April when it started to rise again to produce a second but much lower peak about the middle of the month. The infestation dropped once more in numbers but only for about a fortnight. It then showed a rapid increase comparable with that of February and March and attained its third peak, the second high one for the year, at the end of May. From this time onwards, there was a steady reduction in the number of larvae present on the cattle until about the third week in July when further warbles were seldom discovered.

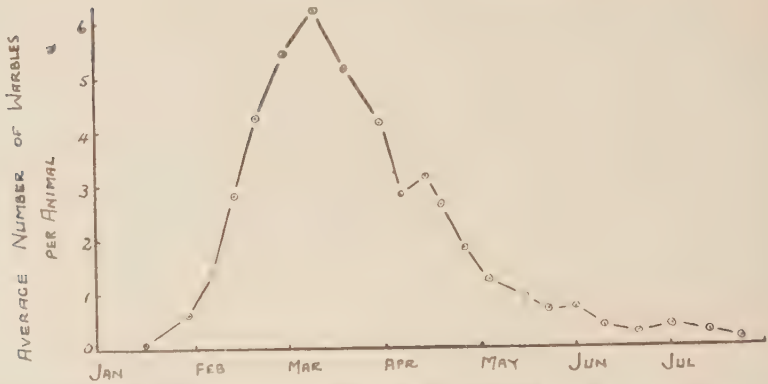


Fig. 5.—Type B. Seasonal incidence of larvae of *H. lineatum* and *H. bovis* in the vertebral region of their host.

Type B. The seasonal curve in the appearance of warbles exhibited by Type B resembled essentially that already described for Type A, except that the third peak is absent (fig. 5). As in the case of Type A, the first and second peaks occurred about the beginning of March and mid-April, respectively. Thereafter, the degree of infestation continued to decline throughout May and June, and apart from an occasional specimen, no larvae were found on the cattle in July.

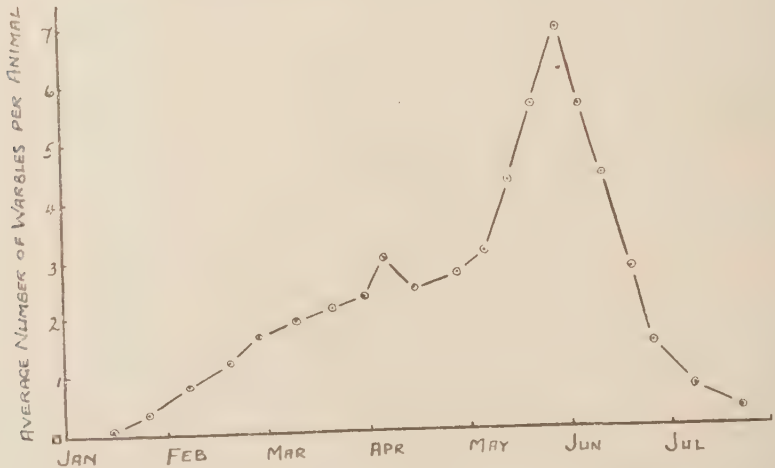


Fig. 6.—Type C. Seasonal incidence of larvae of *H. lineatum* and *H. bovis* in the vertebral region of the host.

Type C. In contrast to Types A and B, the larval population in the case of Type C (fig. 6) never attained a distinct peak in early March. Although the activity in the vertebral region became noticeable about the middle of January, the rate of increase in the infestation remained exceedingly low throughout February and March. A small peak developed about mid-April comparable with that in Types A and B. The population then showed a rapid increase, reaching the highest peak of the season towards the end of May. This was followed by a steady decline in numbers until mid-July, after which hardly any warbles were seen.

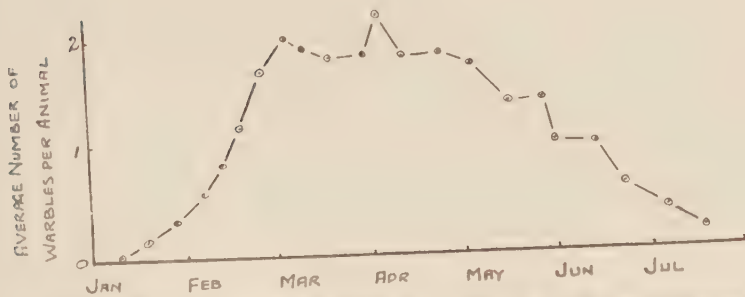


Fig. 7.—Type D. Seasonal incidence of larvae of *H. lineatum* and *H. bovis* in the vertebral region of the host.

Type D. The seasonal infestation exhibited by Type D (fig. 7) was characterised by its lower intensity throughout the season, compared with that shown by Types A, B and C. The duration of the period during which warbles were present was, however, equal to that of the other three types. Type D exhibited two low peaks, one in early March and the other about mid-April, and, in addition, there was a period of slight fluctuations in the degree of infestation towards the end of May and beginning of June. This type of seasonal prevalence was commonly encountered in herds of cattle grazing on the uplands or on exposed pastures at the time when the eggs are deposited by the ox warble flies.

(b) *Investigations to elucidate the factors responsible for the variations.*

A study was made of the significance of these marked variations in the number of warbles present on cattle at different periods from early January until the end of June. All the larvae were carefully removed from the backs of carcasses and flayed hides of animals slaughtered at the abattoir, Pencoed, Glamorgan, weekly from the beginning of February to the end of July in 1945 and the larvae were identified in the laboratory. The results are shown in fig. 8 and it will be seen that the larvae which were recovered up to about the beginning of March belonged to the species *H. lineatum*. As the larval population of *H. lineatum* declined from early March to about mid-May, the numbers of larvae of *H. bovis* increased at an approximately corresponding rate. After the middle of May, the larvae found on the cattle belonged to *H. bovis*. It must be emphasised in this connection, however, that an occasional larva of *H. bovis* was collected on the cattle earlier than the beginning of March and of *H. lineatum* after mid-May but the number in each case was too low for representation in fig. 8 as the graphs are based on the 3,541 warbles identified during the entire period involved.

A consideration of the foregoing data in relation to the larval populations present in the subcutaneous tissues on each side of the spinal column of cattle at different periods between January and late July provides definite evidence on the factors responsible for the four main types of variations (A, B, C, D) already discussed.

Type A. The gradual rise in the degree of infestation throughout January, February and early March, shown by Type A of the seasonal curve, was due to the increasing numbers of larvae of *H. lineatum* reaching the back of the animal during this period (fig. 4). The decline in the infestation from the high peak in the first week in March to the beginning of April was associated partly with the fewer larvae of *H. lineatum* reaching the back and partly with the increasing numbers of larvae of this species which became fully grown and dropped off to pupate. The increase in the infestation responsible for the small peak in mid-April was the result of *H. bovis* larvae arriving at a faster rate than that at which *H. lineatum* larvae were leaving to pupate. Afterwards there was a drop in the infestation for about a week

due to the fact that the majority of *H. lineatum* larvae had now attained maturity and left the host. The gradual build-up in the larval population from about the third week in April to the peak of intensity at the end of May was related to the increasing numbers of *H. bovis* which reached the final stage of migration within the host. The decrease in the infestation following this peak was correlated partly with the gradual decline in the number of *H. bovis* larvae reaching the back of the animal and partly with the ever-increasing rate at which the larvae were emerging to pupate. This type of seasonal incidence is found in localities where *H. lineatum* and *H. bovis* occur in approximately equal numbers.

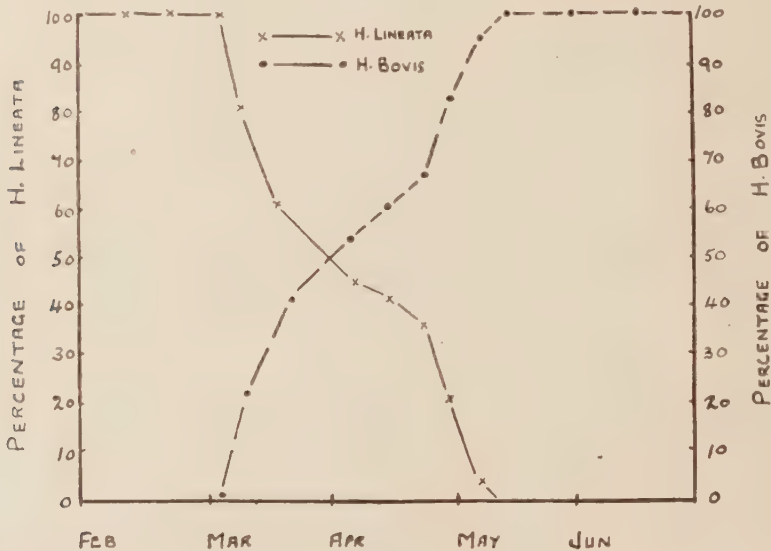


Fig. 8.—Relative prevalence of larvae of *H. lineatum* and *H. bovis* from January to July in the vertebral region of the host.

Type B. The high peak in March and the one of lower magnitude in April, exhibited by Type B (fig. 5) are accounted for in the same way as for Type A (fig. 4). The absence of a third peak in Type B (fig. 5) indicates that *H. bovis* played no significant part in the infestation of the herds involved. It can be inferred, therefore, that *H. lineatum* is the dominant species whenever this particular type of seasonal curve of larval infestation is encountered.

Type C. The absence of a distinct peak in March in Type C was associated with the abnormally low numbers of *H. lineatum* throughout the season (fig. 6). The low peak in April and the high one in May were attributable to the same circumstances as those already given for the corresponding peaks in Type A (fig. 4). In districts where this particular type of larval incidence on herds occurs it can be assumed that *H. bovis* constitutes the predominant species.

Type D. The absence of pronounced peaks in March and May in Type D was correlated with low numbers of both *H. lineatum* and *H. bovis* throughout the season (fig. 7). The fact that this type of seasonal prevalence is only found commonly in the case of herds of cattle grazing on high exposed pastures supports this conclusion. These conditions are not conducive to heavy attacks and it would seem that either few adult flies of both species frequent such areas or that they are not highly persistent in their endeavours to oviposit on the hairs of cattle under such circumstances.

Degree of Incidence of *H. lineatum* and *H. bovis*.

The results of the general survey conducted over several years on the incidence of the ox warble flies in South Wales revealed that both *H. lineatum* and *H. bovis* occur in the area and that no locality is entirely free from either of these species. In order to obtain definite information on the degree of incidence, special investigations were undertaken in 1945 and 1946. All the herds of cattle examined each year in widely separated districts between February and July contained a high percentage of cattle showing varying degrees of infestation by both *H. lineatum* and *H. bovis*. All breeds of cattle, including Aberdeen Angus, Ayrshire, Friesian, Guernsey, Hereford, Jersey, Lincoln Red, Red Poll, Shorthorn and Welsh Black were found liable to carry heavy infestations of both species.

(a) Degree of incidence based on records of warbles on cattle in early March.

Early in March, 1945, 372 cattle exceeding three years of age in the Pencoed—St. Mary Hill areas, Glamorgan, were examined for the presence of larvae in the subdermal tissues of their backs. The total number of warbles actually present on each infested animal was also recorded. The results were as follows :—

Total number of cattle examined	..	372
Total number of cattle infested	..	278
Percentage of cattle infested	74.73
Average number of larvae per animal—		
For all cattle examined	3.8
For infested cattle only	5.0

In the same period, 306 young cattle varying in age from eight months to two and a half years at the same centres were also examined and the number of warbles recorded. The results showed :—

Total number of cattle examined	..	306
Total number of cattle infested	..	267
Percentage of cattle infested	87.25
Average number of larvae per animal—		
For all cattle examined	9.4
For infested cattle only	10.8

It will be noted from these figures that the degree of incidence in Glamorgan, based on the number of warbles present on cattle in early March, 1945, is high, over 80 per cent. of the 678 animals of all ages included in the survey, being infested. These figures, when taken by themselves, also indicate that cattle under three years old are more liable to infestation than those over this age, the difference, on an average, in the number attacked being approximately 12 per cent. Further, the figures indicate not only that young cattle are more subject to become infested but also carry heavier infestations, the intensity of attack being 10.8 warbles per animal compared with 5.0 on cattle over 3 years old. It must not be concluded from these results, however, that the flies always show a definite preference for young cattle. It was found by the writers in the general observations made over many years that young cattle are more susceptible to attack but that in several of the cases investigated, the higher incidence could be associated with differences in the management of the two groups of cattle. The cattle in the older group consisted almost invariably of milking cows and received far more care and attention than the younger cattle which were often kept on open pastures with no access to rivers, ponds or shade of any kind during the height of activity of the ox warble flies. It would seem that conditions favouring oviposition rather than preference for any particular age group of animals may frequently decide the degree and intensity of attack by both *H. lineatum* and *H. bovis*.

Another series of counts of warbles was made on herds selected at random in the same localities in the first week of April, 1945. Altogether 412 cattle of all ages were carefully examined on this occasion, the majority being milking cows over three years old. The result revealed :—

Total number of cattle examined	..	412
Total number of cattle infested	..	293
Percentage of cattle infested	..	71.12
Average number of warbles per animal—		
For all animals examined	..	4.1
For infested animals only	..	7.0

These figures show that the proportion of cattle harbouring warbles was much lower than that earlier in the season, the rate of infestation being about 71 per cent. in April compared with 80 per cent. for the two age groups in March. It was observed, however, that of the 119 cattle (29 per cent.) free from attack when examined in April, an appreciable proportion exhibited the characteristic apertures made by the larvae for respiration. These larvae had already attained maturity and emerged to pupate.

(b) *Degree of incidence based on records of warbles during the entire season.*

In order to obtain further data on the degree of incidence in South Wales, all the larvae present in the subcutaneous tissues of the back of each of 171 cattle were recorded each week for the entire "warble season" in 1945, a period extending from early January to end of July. The absolute infestation figures, that is, the total number of larvae which reached the vertebral region of each animal during the entire season, were calculated from the data for the weekly warble counts and the average time spent by the warbles in the backs of cattle (42.5 days, see p. 644). The results are summarised in Table III where it will be noticed that the cattle were arranged in groups according to the number of larvae recorded on them during the season.

TABLE III.

Absolute Infestations of Larvae of *H. bovis* and *H. lineatum* in 1945.

Group	Total No. of Cattle in each Group	Average Infestation in each Group
0	15	Nil
1-5	43	4.2
6-10	42	7.6
11-15	27	12.4
16-20	13	17.5
21-25	12	22.2
26-30	12	29.0
31-35	3	34.6
36-40	Nil	Nil
41-45	3	43.6
Over 46*	1	74.0

* This was a roan-coloured Shorthorn cow about nine years old in a poor condition and the 74 larvae had left it to pupate by 26th March, 1945.

Of the 171 cattle involved in this series of observations, only 8.4 per cent. remained free from infestation throughout the whole season while about 63 per cent. had, on an average basis, an absolute infestation between 4.2 and 12.4 warbles per animal. Approximately 21 per cent of the cattle showed an absolute infestation between 17.5 and 29.0 larvae for each animal and 4.0 per cent. of them over 30 larvae per beast.

Further information on the incidence was obtained in 1946 when weekly counts were repeated over a similar period. The counts were made mainly on the same cattle but only on 125 animals in contrast to the 171 in 1945. The results for 1946 are presented in Table IV.

TABLE IV.

Absolute Infestations of Larvae of *H. bovis* and *H. lineatum* in 1946.

Group	Total No. of Cattle in each Group	Average Infestation in each Group
0	23	Nil
1-5	36	2.7
6-10	18	6.4
11-15	11	12.3
16-20	6	17.8
21-25	6	22.5
26-30	20	27.5
31-35	3	34.3
36-40	1	40.0
41-45	Nil	Nil
Over 46	1	47.0

Of the 125 cattle included in this survey, 18.4 per cent. showed no infestation at any time in 1946. Approximately 52 per cent. had, on an average basis, an absolute infestation between 2.7 and 12.3 warbles per animal whilst about 25 per cent. of the cattle carried an absolute infestation between 17.8 and 27.5 larvae and 4.0 per cent. over 30 larvae per animal.

(c) *Degree of incidence based on records of warbles on cattle slaughtered at a local abattoir.*

Valuable information on the degree of incidence in South Wales and on the relative prevalence of the two species, *H. bovis* and *H. lineatum*, was obtained by the examination of the carcasses and flayed hides of cattle killed at the abattoir Pen-coed, Glamorgan. These cattle of various ages, colour and breeds came from all parts of Glamorgan and the border districts of the adjoining counties. As already mentioned, the abattoir was visited each week from the beginning of February to about the end of July, 1945, and on each occasion the warbles present on some 24 cattle were removed and later identified in the laboratory. The results obtained were as follows:—

Total number of cattle examined	..	492
Total number of cattle infested	..	321
Percentage of cattle infested	..	65.24
Average number of larvae per animal—		
For all cattle examined	..	7.2
For infested cattle only	..	11.1
Total number of larvae recorded	..	3,562
Total number of larvae identified	..	3,541
Total number of larvae of <i>H. lineatum</i>	..	1,636
Total number of larvae of <i>H. bovis</i>	..	1,905

These figures indicate an incidence on a percentage basis for South Wales of 46.2 for *H. lineatum* and 53.8 for *H. bovis*. The highest infestation recorded on any animal during these studies at the abattoir was 152 warbles on a roan-coloured Shorthorn heifer about one year old and in a poor condition. It was killed on 18th February,

1945, and all the larvae, as was to be expected for this time of the year belonged to the species *H. lineatum*. This number of warbles on a single animal constitutes the highest infestation recorded hitherto in South Wales and Monmouthshire. The next highest infestation was 110 warbles, recorded on 28th March, 1945, on a Hereford heifer about eighteen months old and in a fairly good condition. Approximately 51 per cent. of these larvae belonged to the species *H. lineatum* and 49 per cent. to *H. bovis*.

The results of these studies based on three different methods of technique indicate a very high degree of incidence in South Wales, over 75 per cent. of the cattle examined in 1945 and 1946 being infested. Further, it would seem that both species are approximately equally abundant, *H. lineatum* being possibly somewhat less numerous than *H. bovis*.

Populations in Relation to Topographical Conditions.

It is well known that topographical conditions play a significant part in determining the degree of infestations of farm animals by certain parasitic insects. For instance, flocks of sheep maintained on low-lying sheltered farms are invariably more heavily attacked by the Sheep Maggot Fly, *Lucilia* sp., than those kept on the uplands or exposed pastures. As very little information has hitherto been available on the relation of topographical conditions to the degree of warble infestations on cattle, herds on both lowlands and uplands were examined at frequent intervals during 1945 and 1946 in an endeavour to obtain definite data.

(a) Comparison of the warble infestations of cattle on Upland and Lowland Farms.

It must be emphasised that in these studies the term "Upland Farm" denotes enclosed land situated 350 ft. to 400 ft. above sea level, in an exposed position and with little or no shade provided by trees or high hedges, whereas "Lowland Farm" indicates agricultural land lying below 100 ft. in altitude with abundant shade and shelter.

The infestation of cattle, based on weekly counts of larvae present in their backs, on a typical (a) Upland Farm and (b) Lowland Farm are presented graphically in fig. 9 and it is evident that there was a marked difference in the intensity of the infestation on these two farms. In early March, there were, on an average, 7.5 warbles per animal on the Lowland Farm compared with 1.5 for each animal on the Upland Farm, whilst in the third week in May, the average number per beast was approximately 4 for the Lowland Farm and 1.5 for the Upland Farm. Comparisons of the data obtained from less intensive counts which were made on essentially comparable types of farms in other areas in Glamorgan confirmed these findings.

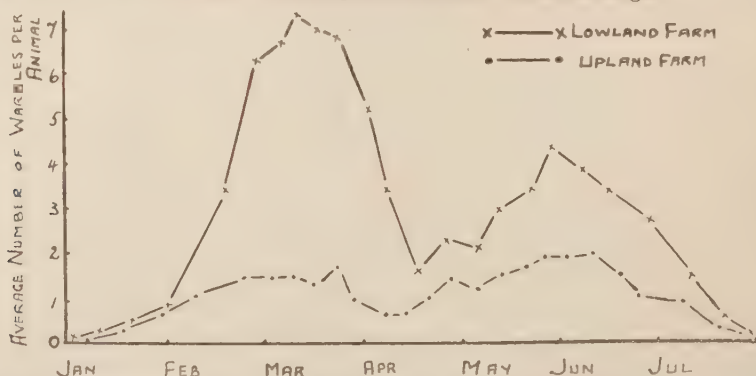


Fig. 9.—Comparative incidence of *H. lineatum* and *H. bovis* in relation to topographical conditions.

The figures representing the average absolute infestations of warbles for 1946 on herds examined on typical Lowland and Upland Farms are given in Table V.

TABLE V.

Warble Infestations on Dairy Herds of Cattle on typical Lowland and Upland Farms in Glamorgan in 1946.

Lowland Farms		Upland Farms	
10.9	Mean 14.47	5.0	Mean 5.52
13.2		5.3	
18.5		5.2	
14.2		6.6	

These results reveal an outstanding difference between the two sets of figures representing the total number of larvae that were found for the whole season on the cattle of the two kinds of farms, the numerical value of the means being 14.47 and 5.52 for the Lowland Farms and Upland Farms, respectively. It is evident from these figures that a definite relationship exists between the degree of infestation of cattle and the topographical conditions.

Cattle of three years old and under this age on some of these two types of farms harboured heavier infestations than those above this age. In view of this fact, comparative counts of the warbles on cattle of the two age groups, which had been maintained at all times under identical conditions, were made in 1946. The results are summarised in Table VI.

TABLE VI.

Comparative Data on the Warble Infestations of Cattle
(a) over 4 years old and (b) under 3 years on typical Lowland and Upland Farms
in Glamorgan in 1946.

Date of Examination	Type of Farm	Class of Cattle	No. of Cattle	No. of Warbles	Average No. Per Animal
Week ending 25-2-46	Lowland ..	Old	90	272	3.0
	Lowland ..	Young	81	918	11.3
Week ending 25-2-46	Upland ..	Old	86	122	1.4
	Upland ..	Young	72	292	4.0

These figures provide some evidence that cattle under three years of age are subject to heavier infestations than those above 4 years old but additional information on this aspect is produced later. They also confirm the results presented in fig. 9 and Table V that the intensity of warble infestations on cattle is related to the topographical conditions of the locality and that herds on the uplands are less liable to severe attacks than those on the lowlands.

(b) *Comparison of warble infestations of cattle on Lowland Farms in relation to shade and water.*

The general observations made by the writers on herds of cattle on a number of farms in South Wales in 1944 and 1945 indicated that altitude alone, at least up to 750 ft. exercises no direct influence on the populations of *H. bovis* and *H. lineatum*, judging by the infestation of cattle, except in so far as it affects certain topographical features. In order to obtain definite evidence, a detailed survey of infestations on the cattle of contrasted types of lowland farms over several years is necessary and

preliminary studies were started in 1946. Four of the farms chosen are well supplied with shade afforded by trees growing in the hedgerows and here and there in the pastures and by water in the form of ponds and streams. The other four farms are more exposed and the fields are not provided with shade and water where cattle can retreat on hot, summer days. The average absolute infestations of warbles on the cattle in 1946 on the eight farms are given in Table VII.

TABLE VII.

The Average Absolute Infestations of Larvae of *H. bovis* and *H. lineatum* on Individual Cattle on Eight Lowland Farms,
(a) Four with ample Shade and Ponds and (b) Four without much Shade or Ponds.

Farms with Shade and Ponds		Farms without Shade and Ponds	
10.3	Mean = 9.1	17.5	Mean = 13.5
9.3		14.3	
9.0		11.3	
7.8		11.0	

The results of these preliminary studies suggest that the varying topographical features even on lowland farms situated at essentially identical altitudes produce an appreciable effect on the degree of infestation.

Infestations in Relation to the Age of the Host.

The age of the host, at least in the case of farm animals, is often an important factor in determining the intensity of attack by parasites of various kinds but hitherto no definite information in this connection has been available regarding infestations by *H. bovis* and *H. lineatum*. A series of observations in this connection were made on cattle of varying ages grazing under the same topographical and climatic conditions in the Vale of Glamorgan. The cattle were separated into two classes, (a) young cattle, that is, animals not more than 2½ years of age and (b) old cattle, that is, animals over 3½ years of age. The results obtained are recorded in Table VIII.

TABLE VIII.

Comparative Data on Infestations of *H. bovis* and *H. lineatum* on Cattle (a) Under 3 years of age and (b) Over 3 years of age.

Date of Examination	Class of Cattle	No. of Cattle	No. of Warbles	Average No. of Warbles per Animal
Week ending 22-2-45	Young	81	918	11.3
	Old	90	272	3.0
Week ending 10-3-45	Young	69	555	8.0
	Old	90	332	3.7
Absolute Infestations	Young	60	1,376	22.9
	Old	78	674	8.6

The data in Table VIII shows that the age of the host plays an important part in deciding the degree of attack, the infestations being far greater in cattle under three years old than in those over this age. The figures for the absolute infestations, that

is, the total number of larvae which reached the backs of the cattle during the whole season, stress this fact more markedly than those for the infestations of a particular period.

Counts of the larvae in the back of each animal in herds composed of cattle varying in age from approximately eight months to seven years and over, were made in the Bridgend area of Glamorgan in the last week of February and the first week of March, 1945, to ascertain whether the intensity of attack is in direct relation to age. The results are given in Table IX.

TABLE IX.

Comparative Data on Infestations by *H. lineatum* and *H. bovis* on Cattle of different Ages from eight months to seven years and over at the Time of Oviposition.

Approximate Ages of Cattle	No. of Cattle	Total No. of Warbles	Average No. of Warbles
8-12 months ..	42	372	8.8
13-18 months ..	54	606	11.2
19-24 months ..	68	674	9.9
25-30 months ..	44	440	10.0
			Mean = 10.0
4 years	57	240	4.2
5 years	46	174	3.8
6 years	52	230	4.4
7 years and over	62	242	3.9
			Mean = 4.1

The figures for the average number of larvae recorded for each animal, shown in the final column of Table IX, indicate that the degree of infestation is not retrogressively proportional to increase in the age of the host. The 425 cattle examined fall into two distinct groups, one consisting of animals below three years of age with a mean value of 10.0 for their larval infestations and the other of animals above this age with a value of 4.1. In both age groups, the infestations show little variation from their respective means, though the latter are strikingly different. It would seem from these results that there is an age limit, above which the animal is less liable to attack, judging by the number of larvae reaching the final stage in their migration within the body of the host. It is not possible as yet to postulate any feasible explanation for this comparative immunity or resistance to attack once the animal attains the age of about three years.

In 1946, weekly counts of warbles on sixteen dairy cattle on a farm at St. Mary Hill, Glamorgan, were made from January to July, inclusive. This farm was chosen since eight of the cattle in the herd were heifers (first calvers) between two or three years old whilst the remainder were cows not less than four years of age at the time of oviposition. Both groups of cattle had been grazing the same pastures the previous season when ox warble flies were active. These cattle had not been treated to prevent oviposition or to destroy the larvae. The results obtained are presented graphically in fig. 10.

The seasonal infestation curve for the young cattle shows a marked peak in the earlier part of March and two less pronounced peaks in April and May, respectively. The critical examination of representative samples of the mature larvae made at intervals throughout the season revealed that the March peak was composed of *H. lineatum* and that in May of *H. bovis*. The infestation curve for cattle over four years old exhibits in the earlier part of the season a slow but gradual rise culminating in a slight peak for *H. lineatum* early in March and later towards the end of May in a relatively high peak of short duration for *H. bovis*. A further striking feature of these curves is the much higher population of larvae on the younger group of cattle throughout the whole period from the beginning of January to August, except for a few days in the latter part of May.

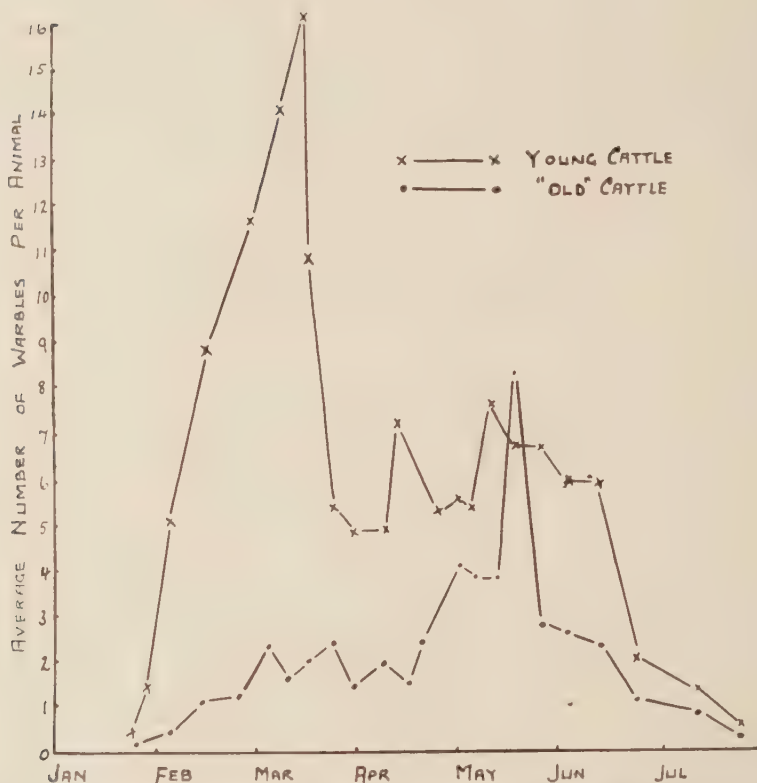


Fig. 10.—Comparative incidence of *H. lineatum* and *H. bovis* in relation to the age of the host.

These results, besides providing further evidence that cattle after passing three years of age become less liable to heavy infestations of warbles, show that those under this age limit are highly susceptible to serious attack. Proof that a critical age does exist in the life of cattle to serious infestations by ox warble flies is of considerable practicable importance as progressive farmers could adopt measures whereby the younger cattle are given preferential treatment in the choice of pastures at the time of oviposition by the flies. Certain fields on most farms offer more protection than others against attack on account of their exposed positions to the prevailing winds or to the presence of ponds, rivers or shade from the sun.

Experiments on Direct Control.

The method of controlling warble flies practised in this country for several years prior to the 1939-45 World War included the use of a derris wash prepared in accordance with the specifications laid down in "The Warble Fly (Dressing of Cattle) Order of 1936", an Order which was withdrawn in 1940 as derris became unprocurable. In 1946, it was decided by the writers to test the value of dichloro-diphenyl-trichlorethane (DDT) and benzene hexachloride (BHC), both having already been used with considerable success against certain other animal pests. They were tested in two distinct series of experiments, (a) as larvicides to kill the larvae in the backs of the cattle and (b) as dressings to prevent oviposition by the adult flies on cattle.

(a) *Comparative tests on the value of DDT and BHC for the destruction of larvae of H. bovis and H. lineatum.*

The following insecticides were tested at different concentrations :—

1. A DDT dust containing 5 per cent. DDT and kaolin as a carrier.
2. A DDT emulsion (proprietary preparation) diluted for use with water to give a fluid containing 1 per cent. DDT.
3. A DDT emulsion consisting of DDT (8.65 per cent.) 16.7 parts, benzene 20 parts, sodium hydroxide 1 part, oleic acid 7.3 parts, casein 1 part and water 54.0 parts, diluted for use with water to give a fluid containing 1 per cent. DDT.
4. A DDT emulsion consisting of DDT (86.5 per cent.) 10 gr., benzene 12 cc., commercial wetting agent (1 part in 1,920 parts water) 4 cc. and 74 cc. of water, diluted for use with water to give a fluid containing 5 per cent. DDT.
5. A DDT emulsion similar to No. 4 but for application diluted with water to give a fluid containing 2 per cent. DDT.
6. A BHC dust containing 2 per cent. actual γ -isomer.
7. A BHC water suspension, diluted with water to give a fluid containing 1 per cent. γ -isomer.

The DDT and BHC dusts (Nos. 1 and 6) were applied by means of a small hand-duster and then rubbed in so as to ensure contact between the insecticides and the embedded larvae. The DDT emulsions Nos. 2 and 3 were applied to the back of an infested animal with a piece of cloth thoroughly soaked in the preparations. Emulsions Nos. 4, 5 and 7 were applied with the aid of a hypodermic syringe, the needle of which had previously been removed. The nozzle of this syringe was sufficiently small to be inserted into the breathing hole made by the larva in the skin. 1 cc. of the emulsion was injected into the cavity surrounding each of the larvae, thus ensuring direct contact with the warble. The results of the different treatments are presented in Table X.

TABLE X.

Results of Experiments on the Value of DDT and BHC Preparations for the Destruction of Larvae of *H. bovis* and *H. lineatum*.

Treatment	No. of Warbles Treated	No. of Larvae after Treatment		
		Dead	Alive	Already Emerged
1. DDT Dust at 5%	82	8	60	14
2. DDT Emulsion (P.P.) at 1% ..	106	6	82	18
3. DDT Emulsion at 1%	116	5	87	24
4. DDT Emulsion at 5%	109	7	89	13
5. DDT Emulsion at 2%	98	6	78	14
6. BHC Dust at 2%	96	6	73	17
7. BHC Water Suspension at 1% ..	84	7	66	11
No treatment	95	6	76	13
No treatment	124	9	91	24
No treatment	99	8	79	12

At first, it would seem from these results that all the treatments were successful in destroying some of the larvae but fortunately three lots of cattle had been kept as untreated controls. The records of the warbles on these untreated cattle show that a small percentage of the larvae present on them had also died. The death of these larvae as well as those on the treated cattle may have been effected by the cattle rubbing their backs against low hanging branches of trees present in the hedges

around the pastures. The death of the larvae, on the other hand, may have been caused by crows or jackdaws in their attempts to dislodge them for feeding purposes. These birds have often been seen by the writers standing on the backs of cattle pecking at the warbles embedded beneath their skin.

It is evident from the results presented in Table X that DDT and BHC, at least in the forms and at the concentrations used in these experiments, are not lethal to the larvae of *Hypoderma* spp. in their final stages in the subcutaneous tissues in the vertebral region of the host, even when applied in such a manner as to ensure direct contact between the insecticides and the warbles. It is also noteworthy that no ill-effects were produced by the treatments on the general health, skin or hair of the treated cattle.

(b) *Comparative tests on the value of DDT and BHC for the prevention of oviposition by H. bovis and H. lineatum.*

Two DDT emulsions were tested in these experiments:—

1. A DDT emulsion diluted with white spirit, obtained from the "cracking" of petroleum, to give a fluid containing 1 per cent. DDT.
2. A DDT emulsion consisting of DDT (86.5 per cent.) 16.7 parts, benzene 20 parts, sodium hydroxide 1 part, oleic acid 7.3 parts, casein 1 part and water 54.0 parts, applied after dilution with water to give a fluid containing 1 per cent. DDT.

These emulsions were applied to the fore and hind legs as well as the flanks or sides of the individual cattle by means of an automatic hand-sprayer, that is, to the parts where the eggs are normally deposited by the flies. Approximately one pint of the diluted emulsion was sprayed on every animal on each occasion. Emulsion No. 1 was used on cattle at Ruthin Farm, St. Mary Hill, and Emulsion No. 2 on cattle at Bryncwytyn Farm as well as The County Demonstration Farm, Pencoed, Glamorgan.

It had been hoped to apply these insecticides at fortnightly intervals in 1945 from mid-May until mid-August but unfortunately, owing to the delay in obtaining suitable preparations from the manufacturers, the applications were made only on three occasions, 18th July, 3rd August and 18th August. The method adopted for assessing the value of these treatments was to make weekly counts of the warbles in the backs of the experimental animals from early January to mid-July, 1946. In order to determine the effects of these treatments as accurately as possible, only the figures for the absolute infestations of *H. bovis* are considered here. To include the data for the infestations of *H. lineatum* would be misleading as the adults of this species are active considerably earlier than those of *H. bovis*. The results obtained are summarised in Table XI.

TABLE XI.

Results of Experiments on the Value of DDT Emulsions for the Prevention of Oviposition by *H. bovis*.

Treatment	Farm	Average No. of Larvae per Animal		Reduction in Average No. of Larvae on Treated Animals
		Untreated	Treated	
1. DDT Emulsion .. (White Spirit) at 1%	Ruthin	6.0	0.8	5.2
2. DDT Emulsion .. (Benzene) at 1%	Demonstn.	9.1	5.9	3.2
Ditto	Bryncwytyn	6.4	0.7	5.7
Average for the 3 farms		7.2	2.1	4.7

The figures for the reduction in the average number of warbles on the treated cattle, shown in the final column of Table XI, indicate that both forms of DDT emulsions when sprayed over the flanks and legs of the animals in July and August, 1945, had been effective in preventing serious larval infestations of *H. bovis* on the three experimental herds in 1946. The actual reduction in the degree of infestation effected by the insecticides amounted to 65 per cent. when applied during the oviposition period in the form of sprays at 1 per cent. concentration of DDT over the parts of the animal where the eggs are normally deposited. It is realised, however, that further and more extensive experiments must be conducted over several seasons before final conclusions can be reached and that for the control of both *H. lineatum* and *H. bovis* the applications must be made at regular intervals, possibly every 14 to 21 days, from mid-May to mid-August.

Summary.

A historical account of the Ox Warble Flies, *Hypoderma lineatum* and *H. bovis*, is given and the economic importance of the two species is discussed. Certain aspects of their biology and control have been studied over a period of several years in South Wales culminating in intensive investigations in the years 1945 and 1946.*

The adults of *H. lineatum* emerge from their puparia in early May and those of *H. bovis* a month later. The adults of both species in their persistent endeavours to oviposit, worry cattle, whenever the weather is sunny and calm, from the end of May to early September but they tend to avoid the vicinity of water and shade.

The degree of incidence is high in Britain, at least in South Wales, judging by the larval populations present in the backs of cattle. Over 80 per cent. of the cattle included in the surveys conducted in 1945 and 1946 harboured infestations of varying intensity; in one case 152 larvae were recorded on a Shorthorn heifer.

The larvae spend, on an average basis, 42.5 days in the subcutaneous tissues of the backs of their hosts. They begin to depart in order to pupate much earlier than is normally assumed to be the case in Britain, more particularly in Worcestershire, where the proportion of larvae pupating prior to March 28th was as low as 1.4 per cent. compared with over 20 per cent. in the present studies.

The marked variations commonly observed in the seasonal incidence of the larvae in their final instars on cattle are correlated with the relative prevalence of the two species in different districts.

The incidence of both species, judging by the intensity of infestations, is associated with the topographical conditions of the locality. For instance, herds on upland pastures are generally less liable to severe attacks than those on lowland farms.

Cattle under three years old normally carry much heavier infestations of the larvae of both species than those exceeding this age when kept under identical conditions during the oviposition period.

Diphenyl-dichloro-trichlorethane and benzene hexachloride preparations are ineffective for destroying the larvae, at least in their final instars, but emulsions of the former insecticide when applied in the form of a spray to the legs and flanks of cattle during the period of oviposition proved highly promising and warrant further investigations.

References.

- BLAGOVESHCHENSKII, D. I. & PAVLOVSKIĬ, V. N. (1930). Zur Biologie und Bekämpfung von *Hypoderma bovis* Deg.—Rep. appl. Ent., Leningrad, 4, p. 371. (Rev. appl. Ent., (B) 19, p. 115.)

* The delay in the publication of the results of this work is largely due to the departure of one of the writers shortly afterwards to take up his present appointment in the University College of the Gold Coast.

- BRAUER, F. (1863). Monographie der Oestriden. Vienna.
- BRAUER, F. (1875). Beschreibung neuer u. ungenügend bekannter Phryganiden u. Oestriden.—Verh. zool. bot. Ges. Wien, **1875**, p. 75.
- BRAUER, F. (1890). Ueber die Feststellung des Wohnthieres der *H. lineata*, Villers.—Verh. zool. bot. Ges. Wien, **40**, p. 75.
- CARPENTER, G. H., HEWITT, T. R. & REDDIN, T. K. (1914). The Warble Flies.—J. Dep. Agric. Ire., **15**, p. 105.
- CARPENTER, G. H. & STEEN, J. W. (1908). The Warble Fly. Experiments on cattle as to its treatment and life history.—J. Dep. Agric. Ire. **8**, p. 277.
- CLARK, B. (1797). Observations on the genus *Oestrus*. Trans. Linn. Soc. Lond., **3**, p. 289.
- CLARK, B. (1815). An essay on the Bots of horses, and other animals. London.
- DAVIES, W. M. (1930). The control of Warble Flies in North Wales.—J. Minst. Agric., **37**, p. 862.
- GAUT, R. C. (1930). Ox Warble Fly. Report on the demonstrations and experiments in 1930.—47 pp. Worcester, Agric. Educ. Sub-Comm.
- GAUT, R. C. & WALTON, C. L. (1929). Ox Warble Fly. Report on the demonstration and experiments carried out in Worcestershire in 1928 and 1929.—26 pp. Worcester, Agric. Educ. Sub-Comm.
- DE GEER, C. (1776). Mémoires pour servir à l'Histoire des Insectes, **6**, p. 297.
- HAWDEN, S. (1915a). Warble Flies. A further contribution on the biology of *Hypoderma lineatum* and *H. bovis*.—Parasitology, **7**, p. 331.
- HAWDEN, S. (1915b). Warble Fly Experiments.—Amer. vet. Rev., **47**, p. 453.
- HINRICHSSEN (1888). Ueber einen neuen Parasiten im Rückenmarkskanal des Rindes.—Arch. wiss. prakt. Tierheilk., **14**, p. 219.
- JOLY, N. (1846). Recherches . . . sur les Oestrides . . .—Ann. Sci. phys. nat. Lyon, **9**, p. 157.
- LATREILLE, P. A. (1825). Familles naturelles du Règne animal. Paris.
- MACDOUGALL, R. S. (1934). Ox Warble Flies.—Trans. Highl. agric. Soc. Scot., **1934**, repr., 90 pp.
- ORMEROD, E. A. (1885–95). Reports of Observations of Injurious Insects. London. 1884–94.
- DE RÉAUMUR, R. A. F. (1738). Mémoires pour servir à l'Histoire des Insectes, **4**, p. 503.
- RILEY, C. V. (1892). The Ox Bot in the United States.—Insect Life, **4**, p. 302.
- VALLISNIERI, A. (1733). Opere (fisico-mediche . . .), **1**, p. 28.
- DE VILLERS, C. J. (1789). C. Linnaei Entomologia Faunae Suecicae . . ., **3**, p. 349.
- WALTON, C. L. (1925). Notes on Warble Flies in North Wales.—Welsh J. Agric., **1**, p. 195.
- WARBURTON, C. (1922). The Warble Flies of Cattle, *Hypoderma bovis* and *H. lineatum*.—Parasitology, **14**, p. 322.
- The Warble Fly (Dressing of Cattle) Order of 1936.—S.R.O. 1936, No. 71. London, 1936.

VARIETAL DIFFERENCES IN THE SUSCEPTIBILITY OF PEAS TO ATTACK BY THE PEA MOTH, *LASPEYRESIA NIGRICANA* (STEPH.).

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The existence of variations in susceptibility to an insect pest in different varieties of the same plant has long engaged the attention of biologists. These differences form the basis of the production of new varieties of crop plants showing increased resistance to the pest concerned. Considerable success in this direction has been achieved by using methods of selective breeding and varietal hybridisation, particularly in the United States and Africa; in Great Britain these problems have received comparatively little attention.

The existence of variations in susceptibility to attack by the Pea Moth, *Laspeyresia nigricana* (Steph.), between different varieties of peas (*Pisum sativum* L.) is indicated by the work of Nicolaisen (1928) in Germany. This worker showed that quickly maturing varieties of peas if sown early, usually escaped the greater part of the Pea Moth attack and that the later a variety came into flower and the longer its flowering period, the greater was the attack that ensued. Where the flowering periods of different varieties were similar, those with the denser and more bushy growth showed the most damage. Nicolaisen was not able, however, to assess the relative importance of length of flowering period and foliage density in determining attack. Hanson and Webster (1936) in the United States showed that those peas which were maturing throughout the whole flight period of the moth suffered the heaviest attack.

It is widely known by horticulturalists in Great Britain that quick maturing varieties of peas, if sown sufficiently early, escape much of the severity of Pea Moth attack. The writers (1948) have confirmed this experimentally with the variety Thomas Laxton.

The present investigation was designed to test the validity of these conclusions and to attempt to record and measure the factors to which possible differences in susceptibility could be attributed. The experiments were carried out at Harlow, Essex, and comprised two trials on adjoining sites in each of which the same six varieties of peas were grown.

Varieties Tested.

The varieties grown were as follows :—

- | | |
|----------------------------|-----------------------------|
| 1. Kelvedon Wonder (K.W.). | 4. Lincoln Blue (L.B.). |
| 2. Foremost (F.). | 5. Harrison's Glory (H.G.). |
| 3. Onward (O.). | 6. Gladstone (G.). |

These were chosen to include early maturing types (Kelvedon Wonder and Foremost), late maturing types (Lincoln Blue and Gladstone) and those of intermediate character. The varieties also exhibited a wide range of haulm length from dwarf (Kelvedon Wonder) and semi-dwarf (Foremost, Onward and Harrison's Glory) to tall (Lincoln Blue and Gladstone). In addition, two of the varieties are normally grown for harvesting as dried peas (Lincoln Blue and Harrison's Glory), whilst the remainder are mostly grown for consumption as 'green peas', thus permitting comparison between the two types. For brevity, the varieties will henceforth be referred to by their initials as shown in the above list.

During the course of the investigation, records were taken of certain botanical features of each variety which, it was thought, might be related to the intensity of attack. Records of these features are summarised in Table I and illustrate the varietal characteristics outlined above.

*Now with the Empire Cotton Corporation, Nigeria.

TABLE I.
Rates of maturation and haulm length in the different varieties.

Variety	Trial No.	Number of leaf axil bearing 1st flower	Days after drilling to :			Length of haulm at maturity, ins.	Days from full flower to 'green picking'
			1st flower	Full flower	'Green picking' stage		
1. Kelvedon Wonder ..	1	8-9	63	69	110	25	41
	2	7	52	59	84	18	25
2. Foremost	1	7	56	63	110	36	47
	2	6-7	48	63	84	30	21
3. Onward	1	11-12	77	83	112	30	29
	2	11-13	61	66	98	22	25
4. Lincoln Blue ..	1	14-16	83	89	128	44	39
	2	12-14	61	68	103	48	35
5. Harrison's Glory ..	1	12-13	77	83	112	31	29
	2	12-14	61	63	98	22	28
6. Gladstone	1	13-15	83	100	128	48	28
	2	13-14	65	70	103	54	33

Experimental Layout.

The first trial was sown on 29th March and the second 5 weeks later, on 3rd May. The site chosen adjoined land on which peas had been severely damaged by Pea Moth in the previous year, in order to ensure a high level of attack.

In each trial, all six varieties of peas were grown and there were four randomised replicates of each variety, *i.e.* four blocks each of six plots in each trial. Individual plots were 15 yards square, separated from each other on all sides by two yards of bare ground. Each trial occupied about one and a half acres. The first trial was placed ten yards from the hedge in order to minimise possible effects of adjoining shelter on subsequent attack. The second trial, on adjacent land, had the same relative position to the source of moths as the first.

Plant Populations.

The seed was sown by a hand drill in rows 18 inches apart and a seed rate of about eight seeds per foot of row was obtained in all cases. Germination was good in all plots but the initial plant populations were somewhat reduced by the depredations of pea weevils (*Sitona* sp.) and wild birds, and by late spring frosts. When the plants in each trial were well established, population counts were made on all plots; the average population per foot of row for each plot and the mean for each variety is given in Table II. The mean population on the first trial varied from 3.3 plants per foot of row in variety K.W. to 6.5 plants in variety L.B. In the second trial the highest and lowest populations were shown by varieties L.B. and G. with 6.7 and 2.7 plants per foot of row respectively. The importance of differences in the replicate plot values is discussed in the section dealing with plant cover.

TABLE II.
Plant populations in both trials (Plants per foot of row).

Replicates :		A	B	C	D	Mean	
1. Kelvedon Wonder	2.2	3.6	4.7	2.6	3.3	Trial I.
2. Foremost	4.5	5.1	3.9	3.7	4.3	
3. Onward	7.9	4.5	4.7	5.1	5.5	
4. Lincoln Blue	8.0	6.5	5.3	6.1	6.5	
5. Harrison's Glory	5.1	5.2	4.1	3.7	4.3	
6. Gladstone	5.9	7.1	5.0	6.1	6.0	
1. Kelvedon Wonder	2.7	5.6	4.4	3.9	4.1	Trial II.
2. Foremost	3.6	4.7	3.3	3.1	3.7	
3. Onward	5.9	5.1	5.2	3.0	4.8	
4. Lincoln Blue	6.6	7.7	6.9	5.5	6.7	
5. Harrison's Glory	5.6	3.4	2.9	4.3	4.0	
6. Gladstone	3.3	3.3	2.6	1.6	2.7	

Life-history of the Pea Moth.

A full account of the life-history of the Pea Moth has been published by the writers elsewhere (1948) and only the salient features will be given here. In England, one generation is produced each year; the moths usually begin to emerge in early June, reach maximum numbers in late June and early July and disappear by early August. The 1946 season, however, was late and emergence began in the third week of June and continued until mid-August. Oviposition on pea crops normally only occurs on those which have begun to flower and, although eggs are laid on all portions of the plant, they are usually restricted to the upper regions. The egg hatches in five to eight days and the young larva wanders over the plant until a pod is encountered into which it bores and feeds on the young seeds. The larva is fully grown after four moults and when mature it cuts a way through the pod wall and drops to the ground. Here it spins a tough cocoon at a depth of $\frac{1}{2}$ to 3 inches, in which the winter is passed; pupation occurs the following spring, usually in May.

Period when the Crop is susceptible to attack.

The pea plant is susceptible to attack over a period beginning shortly after the onset of flowering and ending when the last formed pods begin to dry out and the seeds to harden. First-instar larvae will enter pods in all stages of maturity, from the small, flat, newly formed pods to those which are becoming dry and corny. It is doubtful, however, if larvae are capable of entering dry pods and, even should they succeed in doing so, the resulting damage would be small as dry seeds are unsuitable for growth of the larvae.

Estimation of the Attack.

In these investigations the Pea Moth attack was assessed at two stages of plant growth, *viz.*, at the 'Green picking' ('green'), and 'Harvesting' ('dry') stages. In the former case, the seeds were soft and juicy, and the pods mostly well filled, with only the oldest slightly wrinkled or corny. Crops in such a condition are suitable for marketing for the green pea trade. In the harvesting stage, the pods on the lower half of the plant were wrinkled and dry, whilst the remainder were mostly corny with a few green pods at the apex of the plant. At this stage, the crop was fit to be cut or pulled prior to harvesting for seed.

The six varieties behaved as three pairs in relation to their speeds of development, and the members of each pair were sampled on the same day, as they matured at very similar rates. Varieties K.W. and F. matured first, varieties O. and H.G. second and varieties L.B. and G. last.

At each stage of sampling, 25 single plants were taken at random from each plot. These were examined individually and the number and size of larvae and the number of damaged and undamaged seeds recorded for each pod. The records were tabulated serially according to the age of the pods so that the distribution of attack in relation to the age groups of these could be followed. It was, therefore, possible to express the attack on each variety either as the percentage pods infested or, since the plant population for each plot was known, as the larval population per unit area. For the purposes of the initial comparison, the former method was used and in Tables III and IV the percentage pod attack is shown at the 'green picking' and 'harvesting' stages for both trials. The data given are the averages for each set of four plots and significant differences, determined from an analysis of plot replicate values, are appended to the tables. In addition, graphical comparisons of the infestations sustained by the same variety on both trials at both the green picking and the harvesting stages are shown in figs. 1 and 2.

TABLE III.

Percentage pod and seed attack. First trial 'green picking' and 'harvesting' stages.

Variety	Date of first flower	Average No. seeds per pod	'Green picking' stage			'Harvesting' stage		
			Date of sampling	Pods attacked %	Seed attack %	Date of sampling	Pods attacked %	Seed attack %
1. Kelvedon Wonder ..	29/5	6.0	15/7	6.4	1.22	6/8	27.4	10.16
2. Foremost	22/5	4.9	15/7	9.8	2.77	6/8	32.4	16.24
3. Onward	12/6	6.4	17/7	16.3	3.45	9/8	48.1	18.40
4. Lincoln Blue ..	18/6	4.4	2/8	32.8	15.40	14/8	39.8	21.30
5. Harrison's Glory ..	12/6	4.0	17/7	10.8	3.35	9/8	30.2	16.00
6. Gladstone	18/6	6.4	2/8	53.3	16.70	14/8	58.2	27.10

Significant Differences. Percentage Pod Attack.

'Green picking' stage.

G > all others at 1% pt.

L.B. > K.W., F., O. and H.G. at 1% pt.

O. > K.W., F. and H.G. at 5% pt.

No significant differences between K.W., F. and H.G.

'Harvesting' stage.

G. > K.W., F., L.B. and H.G. at 1% pt.; > O. at 5% pt.

O. > K.W. at 1% pt.; F. > K.W. at 5% pt.

No significant differences between F., L.B. and H.G.

TABLE IV.

Percentage pod and seed attack. Second trial 'green picking' and 'harvesting' stages.

Variety	Date of first flower	Average No. seeds per pod	'Green picking' stage			'Harvesting' stage		
			Date of sampling	Pods attacked %	Seed attack %	Date of sampling	Pods attacked %	Seed attack %
1. Kelvedon Wonder ..	24/6	6.1	26/7	19.8	4.8	8/8	27.8	11.7
2. Foremost	20/6	5.1	26/7	22.6	6.73	8/8	22.4	9.9
3. Onward	3/7	6.5	2/8	34.3	11.29	26/8	41.4	20.8
4. Lincoln Blue ..	3/7	4.7	14/8	26.7	12.84	5/9	27.9	15.4
5. Harrison's Glory ..	3/7	4.7	2/8	23.9	9.79	26/8	32.5	17.8
6. Gladstone	7/7	6.4	14/8	25.1	9.25	5/9	29.4	13.8

Significant Differences. Percentage Pod Attack.

'Green picking' stage.

O. > all others at 5% pt.

No significant differences between K.W., F., L.B., H.G. and G.

'Harvesting' stage.

O. > K.W., F., L.B. and G. at 1% pt.

H.G. > F. at 1% pt.

No significant differences between K.W., F., L.B. and G.

Varietal susceptibility to Pea Moth Attack
Green Picking stage

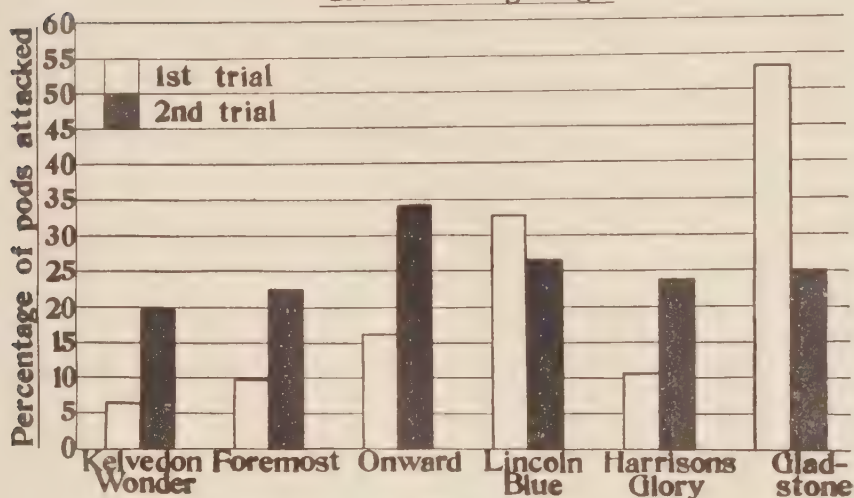


FIG. 1.

Varietal susceptibility to Pea Moth Attack
Harvesting stage

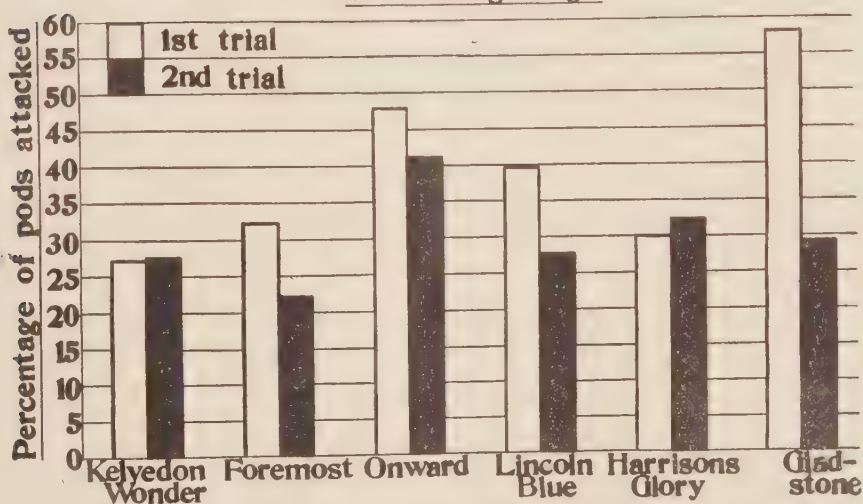


FIG. 2.

Reference to Table III shows that in the first trial the lowest pod attacks at each stage of sampling occurred on varieties K.W., F. and H.G. and in none of these was the attack significantly different. The attack on variety O. at both stages of sampling was greater than on either of the aforementioned three varieties, but at the 'green picking' stage it showed a lower infestation than variety L.B.; at 'harvesting' these positions were reversed. The highest pod infestation at both stages of sampling was incurred by variety G.

In the second trial, the pod infestations show that, at the 'green picking' stage, the attack was almost identical in all varieties with the exception of variety O., where the attack was significantly higher. At the 'harvesting' stage, variety O. was again the most heavily attacked, the remainder suffering a lower and almost equal attack.

A comparison of the infestations on the two trials shows that there was much less variation in attack on the second trial than on the first. The greatest range occurred at the 'green picking' stage of the first trial but the differences were greatly reduced by subsequent attack.

Seed Damage.

In Tables III and IV the percentage of the seed damaged by Pea Moth in each variety is also given. The records show that, in general, the damage is a reflection of the pod infestation. The relationship between seed damage and pod attack when compared in the different varieties at the same stage of sampling is not, however, constant. Thus at the 'green picking' stage, in both trials, the early maturing varieties K.W. and F. show a much lower percentage seed attack for a given pod infestation than in the case of the later varieties L.B. and G. These differences appear to be related to the number of days during which the varieties were exposed to attack. This period was shortest on the early varieties which flowered first and hence in these the larvae had a shorter period in which to damage the seeds before the samples were taken. Consequently the larvae present in the 'green' samples of the early varieties were mostly immature, whilst in the later varieties they were a much older population.

The differences in seed attack between varieties are illustrated by comparing the average number of seeds attacked by a single larva in each case. These values for both sampling stages are listed in Table V. At the 'green picking' stage, the level of damage closely reflected the degree of maturity of the larval population. A high percentage of the larvae had reached maturity at the 'harvesting stage' in all cases and the average number of seeds attacked per larva was very similar for all varieties.

TABLE V.

Relationship between number of seeds damaged and maturity of the larvae at the 'green picking' and 'harvesting' stages.

Variety	'Green picking' stage				'Harvesting' stage			
	Days from full flower to 'green picking'	No. of days exposure	No. of seeds damaged by 1 larva	% Larvae immature	Days from full flower to harvesting	No. of days exposure	No. of seeds damaged by 1 larva	% Larvae immature
<i>Trial I.</i>								
1. Kelvedon Wonder	41	23	1.32	94.3	63	45	2.07	34.7
2. Foremost ..	47	23	1.54	79.0	69	45	2.26	28.1
3. Onward ..	29	25	1.24	89.0	52	48	2.53	15.3
4. Lincoln Blue ..	39	39	2.07	65.4	51	51	2.57	15.5
5. Harrison's Glory..	29	25	1.16	88.8	52	48	2.03	25.0
6. Gladstone ..	28	28	1.74	71.7	40	40	3.05	7.0
<i>Trial II.</i>								
1. Kelvedon Wonder	25	25	1.29	73.0	38	38	2.26	13.6
2. Foremost ..	21	21	1.37	58.2	34	34	2.15	20.4
3. Onward ..	25	25	1.74	60.0	49	49	2.77	8.6
4. Lincoln Blue ..	35	35	2.43	29.4	57	57	2.57	13.1
5. Harrison's Glory..	28	28	1.61	64.5	52	52	2.29	7.6
6. Gladstone ..	33	33	2.31	54.0	55	55	2.84	23.1

Factors that influence Pea Moth Attack.

The data presented above have shown that the incidence of attack varies on different varieties sown on the same date and also on the same variety when sown on different dates. It is, therefore, necessary to inquire into the nature of the factors which determine these differences.

An examination of Table I shows that the varieties chosen required differing lengths of time to reach similar stages of development. In the first trial this resulted in the early maturing varieties K.W. and F. passing part of their susceptible period before the moths were on the wing. The later maturing varieties L.B. and G. were exposed, in this instance, to moths over the whole of their susceptible period, and they suffered a much heavier attack than either of the two former varieties. In the second trial the same two early varieties were exposed to moths over the whole of their susceptible period and suffered a heavier attack than in the first trial (see Tables III and IV). It appears, therefore, that the length of time during which a variety is in a susceptible condition whilst moths are present is very important in determining the intensity of attack. These different periods will, however, be associated with variations in the abundance and activity of the moths and therefore, in assessing the importance of known periods of exposure, account must be taken of these variations.

Another factor which appears to influence the intensity of attack is the degree of shelter provided by the crop. Sweeping has shown that the greatest numbers of moths occur where the crop is most dense. It can therefore be expected that, other conditions being equal, the number of eggs laid in any locality will be in proportion to the number of moths sheltering there and that the intensity of the subsequent attack will be directly related to the number of eggs available. The behaviour of the carrot fly (*Psila rosae* F.) appears to be similarly influenced by shelter for it has been shown that the amount of available shelter both in and around the carrot crop has a marked effect on the distribution of the flies and thereby on the distribution and intensity of damage caused by larvae (see Wright & Ashby, 1946).

In view of the foregoing considerations, information was collected in both trials relating to the period of exposure to moths and to the shelter which each variety provided. In addition, a combined estimate of the abundance and activity of moths over their whole flight period was obtained.

Measurement of certain Factors that influence Attack.

(a) *Period of exposure.*

The period of exposure to attack for each variety was taken as the number of days during the flight period of the moth when the variety was in a condition susceptible to attack. It was indicated earlier that susceptibility commences shortly after the beginning of flowering; for the purposes of this investigation, it was taken as the time when almost all the plants in a crop had begun to flower ('full flower stage'). Susceptibility was taken as having ended when the pods began to dry out and at which time the harvesting stage samples were taken. The flight period of the moth began on 22nd June and lasted until the third week of August. Thus, for each variety, the length of the overlap (in days) between its susceptible period and the flight period of the moth was regarded as its *exposure period*. The values for this period for both trials, and at both stages of sampling are shown in Tables VI to IX, and in fig. 3 are represented by the full lines below the curve.

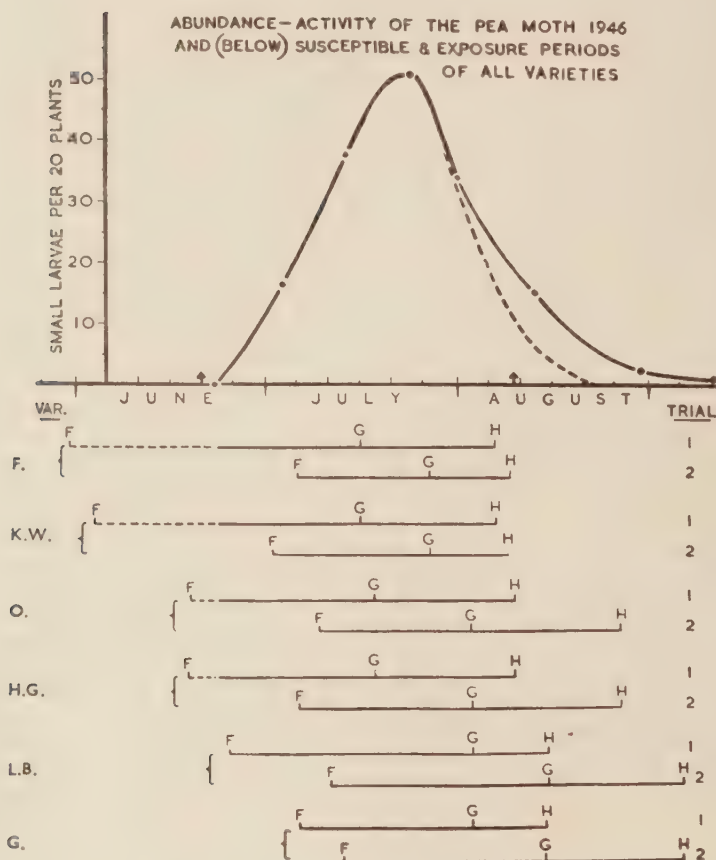


Fig. 3.—Full lines under the curve represent the length of the exposure periods, from full flower (F) to 'green picking' (G) and full flower to 'harvesting' stage (H) for the different varieties.

(b) *Abundance and activity of the moths.*

The number of moths taken in a standard number of sweeps in these trials varied considerably from day to day with the changing activity of the moths. It was considered, therefore, that an estimation of the moth population by this method would not reflect true variations in the numbers present. It was decided to obtain a combined measure of the activity and numbers of the moths over the period concerned. An attempt to record the egg population at regular intervals was discontinued owing to the labour involved and an alternative and quicker method was adopted, based on the numbers of very small larvae (3.5 mm. and less in length), occurring in pod samples taken at bi-weekly intervals from a neighbouring crop of Onward peas. The numbers of small larvae so found in each sample were taken as a reflection of the number of eggs laid, and of the active moths present at a certain preceding date. For each sample, this date appeared to have been about 14 days before sampling, this time being taken up by an incubation period of 7-8 days and not more than a further 7 days to include the age of the largest larvae in this 'small' category. Sampling commenced on 6th July and continued until the end of August, and from the data thus obtained the curve of fig. 3 was constructed, each value being predicated

by 14 days. The first record indicated that moths were active by 22nd June and showed that the peak period of activity occurred between the 20th and 25th of July. These estimates correspond closely with direct information on their emergence and abundance in the field. The curve of fig. 3 can consequently be regarded as the population-activity curve of the moths during their flight period and more closely represents the trends in reproductive potential of the population than data obtained by sweeping. It does not, however, take into account possible inter-varietal variations in mortality among the 1st instar larvae which may have occurred whilst these were wandering in search of pods.

The proportion of the active moth population to which each pea variety had been exposed was then estimated. This was derived from fig. 3 by measuring the area under the curve limited by the particular exposure period and thus represents a summation of daily moth abundance-activity over this period. This summation, termed "Index of Exposure" is shown in Tables VI to IX.

(c) Plant cover.

The density of the pea foliage on the different plots depended on two factors, namely the growth habit of the different varieties and the plant population. In making comparative estimates of plant cover, both factors were therefore taken into account and the following procedure adopted. In the first trial, as each variety reached maturity, four plants of average size were taken from each of two plots and weighed. These were then displayed against a squared background and photographed; the area covered by the plants could then be measured and the average area of foliage per plant obtained. A sample of four plants was taken in the second trial from all replicates at maturity. These were weighed but not photographed and the foliage area per plant calculated from the weight and area relationships obtained for the corresponding varieties in the first trial. With this information and knowing the plant population on each plot, the area of foliage per unit length of row was calculated in each case. This was designated 'Plant cover' and expressed as square inches per foot of row. In this way a comparative measure of the sheltering capacity of each variety was obtained; the values of this measure are also shown in Tables VI to IX.

TABLE VI.

Larval density and factors influencing the level of attack. First trial. 'Green picking' stage.

Variety	Date of full flower	Date of sampling	No. of days exposure	Index of exposure	Plant cover, per foot row sq. in.	Index of susceptibility $\times 10^{-5}$	Larval density per foot row
1. Kelvedon Wonder ..	4/6	15/7	23	1,689	100.45	1.697	2.29
2. Foremost	29/5	15/7	23	1,689	127.19	2.148	2.67
3. Onward	18/6	17/7	25	2,045	177.76	3.635	8.44
4. Lincoln Blue ..	24/6	2/8	39	4,856	201.36	9.778	27.66
5. Harrison's Glory ..	18/6	17/7	25	2,045	160.09	3.274	6.70
6. Gladstone	5/7	2/8	28	4,413	272.84	12.040	26.78

Significant Differences. (All Figures mean of four Plots.)

Larval density.

G. and L.B. > K.W., F., O. or H.G. at 0.1% pt.

O. > K.W. and F. at 5% pt.

No significant difference between K.W., F. and H.G.; O. and H.G.; L.B. and G.

TABLE VII.

Larval density and factors influencing the level of attack. Second trial. 'Green picking' stage.

Variety	Date of full flower	Date of sampling	No. of days exposure	Index of exposure	Plant cover, per foot row sq. in.	Index of susceptibility $\times 10^{-5}$	Larval density per foot row
1. Kelvedon Wonder ..	1/7	26/7	25	3,619	105.91	3.833	6.55
2. Foremost	5/7	26/7	21	3,368	86.94	2.938	4.78
3. Onward	8/7	2/8	25	4,143	222.10	9.202	13.94
4. Lincoln Blue ..	10/7	14/8	35	4,892	379.22	18.551	32.64
5. Harrison's Glory ..	5/7	2/8	28	4,415	191.77	8.467	15.48
6. Gladstone	12/7	14/8	33	4,503	133.18	5.998	8.12

Significant Differences. (All Figures mean of four Plots.)

Larval density.

L.B. > all others at 0.1% pt.

H.G. > K.W. and F. at 0.1% pt., > G. at 1% pt.

O. > F. at 0.1% pt., > K.W. at 1% pt. > G. at 5% pt.

No significant difference between K.W., F. and G.; and O. and H.G.

TABLE VIII.

Larval density and factors influencing the level of attack. First trial. 'Harvesting' stage.

Variety	Date of full flower	Date of sampling	No. of days exposure	Index of exposure	Plant cover, per foot row sq. in.	Index of susceptibility $\times 10^{-5}$	Larval density per foot row
1. Kelvedon Wonder ..	4/6	6/8	45	5,239	100.45	10.53	9.28
2. Foremost	29/5	6/8	45	5,239	127.19	13.33	7.86
3. Onward	18/6	9/8	48	5,527	177.76	19.65	20.98
4. Lincoln Blue ..	24/6	14/8	51	5,832	201.36	23.48	33.86
5. Harrison's Glory ..	18/6	9/8	48	5,527	160.09	17.69	21.02
6. Gladstone	5/7	14/8	40	5,389	272.84	29.41	28.74

Significant Differences. (All Figures mean of four Plots.)

Larval density.

L.B. > K.W. and F. at 0.1% pt., > O. and H.G. at 1% pt.

G. > K.W. and F. at 0.1% pt., > O. and H.G. at 5% pt.

O. and H.G. > K.W. and F. at 1% pt.

No significant differences between K.W. and F.; O. and H.G.; L.B. and G.

TABLE IX.

Larval density and factors influencing the level of attack. Second trial. 'Harvesting' stage.

Variety	Date of full flower	Date of sampling	No. of days exposure	Index of exposure	Plant cover, per foot row sq. in.	Index of susceptibility $\times 10^{-6}$	Larval density per foot row
1. Kelvedon Wonder ..	1/7	8/8	38	5,249	105.91	11.12	9.57
2. Fo. emost	5/7	8/8	34	4,998	86.94	8.69	5.08
3. Onward	8/7	26/8	49	5,477	222.10	24.33	13.20
4. Lincoln Blue ..	10/7	5/9	57	5,278	379.22	40.03	28.06
5. Harrison's Glory ..	5/7	26/8	52	5,749	191.77	22.05	16.63
6. Gladstone	12/7	5/9	55	4,899	133.18	13.10	6.62

Significant Differences. (All Figures mean of four Plots.)

Larval density.

L.B. > K.W., F., O. and G. at 0.1% pt., > H.G. at 1% pt.

H.G. > F. at 1% pt., > G. at 5% pt.

O. > F. at 5% pt.

No significant difference between K.W., F. and G.; K.W., O. and G.; K.W., O. and H.G.

Plant cover.

L.B. > all at 0.1% pt.; O. > K.W., F. and G. at 0.1% pt.; > H.G. at 5% pt.

G. > F. at 1% pt.

No significant difference between K.W. and F.

Larval Density.

In order to compare directly the incidence of attack on all plots, it was necessary to have a direct measure of the populations of larvae. This was readily determined from the number of larvae occurring on the plant samples, and from the plant population per foot of row. In this way the average number of larvae per foot of row was determined for each plot and designated 'Larval density' (see Tables VI to IX). It must be emphasised here that this assessment of 'attack' is different from that given by the percentage pods attacked and is not directly related to it, the reason for this being that larval density is markedly dependent on the pod population which varies with the different varieties whereas the percentage pod attack takes no account of this factor. Assessment of larval density appears to be the best method of comparing the infestations on the various varieties since it gives a combined measure of the attractiveness of the crop and its capacity to be attacked in each case.

The replicated plot values of larval density were analysed statistically and the significant differences between varieties are appended to Tables VI to IX. Certain relationships between the factors measured, namely length of exposure, index of exposure, plant cover and larval density can be seen from these tables:—

- (a) There is no consistent relationship between the length of exposure (in days) and larval density. This is primarily due to differences in moth population during the exposure periods and is readily apparent if the areas under the abundance-activity curve (fig. 3) for similar exposure periods are compared.

- (b) There is a close relationship between the index of exposure and larval density, the former being taken as a combined measure of the abundance and activity of the moths over the period of exposure. Certain discrepancies are, however, apparent for, although within each variety the attack is closely connected with its index of exposure, comparison of the attacks suffered by different varieties with similar exposures does not show a consistent relationship (Table VII, compare at the 'green picking' stage in Trial II varieties L.B. and G. which have very similar indices of exposure but their larval densities are in the ratio of 4 to 1). These and other discrepancies indicate that another factor or factors were also causing variations in attack.
- (c) A consistent relationship is apparent between plant cover and larval density, high plant cover being associated with high larval density and vice versa. Furthermore, as indicated above, when similar exposures produce different attacks, the difference is directly related to a difference in plant cover (in Tables VI and VII compare (i) the 'green picking' stage of variety G. in Trial I with that of variety H.G. in Trial II, and (ii) the 'green picking' stages of varieties L.B. and G. in both trials).

It is thus apparent that the two factors, index of exposure and plant cover are closely associated and exert a joint influence on the subsequent attack. These two factors were combined in the Index of Susceptibility, which for each variety was taken as their product. Its values are listed in Tables VI to IX and each value of the product has been divided by an arbitrary figure (100,000) to reduce it to a convenient size. It is apparent from the tables and from figs. 4 and 5 that this composite factor shows a closer relationship with the subsequent attack than does either of its component factors taken separately.

Analyses of correlations between the percentage pod attack, larval density, the index of exposure and plant cover were made separately and the results compared. Replicated values were available for the two former measurements in both trials and at both stages of sampling. The values for plant cover were replicated only in the

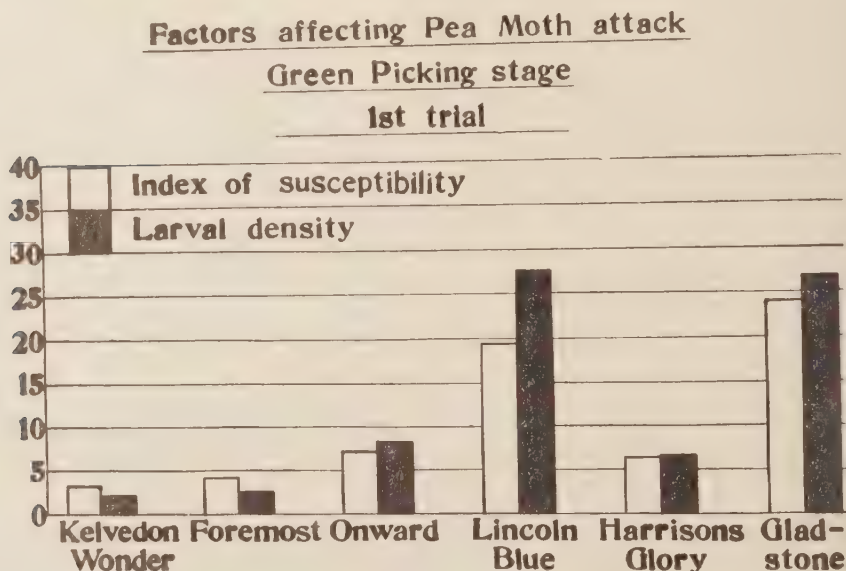


FIG. 4.

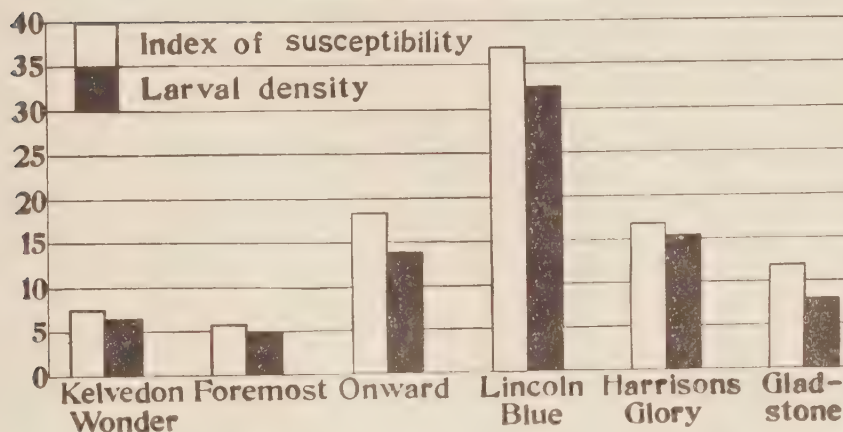
Factors affecting Pea Moth attack**Green Picking stage****2nd trial**

FIG. 5.

second trial at the harvesting stage : since all four plots of each variety were sown together, flowered and were sampled simultaneously, there was no replicate variation in the index of exposure for any one variety. Consequently, analyses were made on the mean values which gave 24 comparisons for each factor ; *viz.*, two trials, six varieties, sampled at 2 stages, ' green picking ' and ' harvesting '.

The results of these analyses may be summarised as follows :—

- Plant cover and the index of exposure both show strong positive correlations with larval density.
- The index of exposure and plant cover alone appear to account for all the variations in larval density and there seems to be no other factor or intrinsic varietal characteristic which effects variations in larval density. In the case of the 6 varieties under trial, those with least plant cover and lowest potential exposure are the least susceptible and vice versa.
- There is a close positive correlation between plant cover and index of exposure. Whilst each has a significant effect on larval density, with that of the index of exposure somewhat the greater, it is not possible to assess their relative importance numerically.
- When the percentage pod attack is compared with the index of exposure there is a significantly positive correlation, but no correlation was evident between the percentage pod attack and plant cover.

Data from other Trials.

The foregoing conclusions relating to the effect of earliness of maturity on the level of infestation were substantiated by data obtained from the Plant Breeding Institute, Cambridge. Here, a series of early maturing Dun and Maple strains have been selected from late maturing commercial stocks of these pea varieties. Their performance was tested in replicated plot trials with the parent commercial stocks in 1946 and 1947. The selections were dwarf types and Table X shows the incidence

of pea moth attack on the seeds and the date of the beginning of flowering—a measure of the earliness of the stocks in each case. Conditions favourable to the Pea Moth in 1947 resulted in the incidence of attack being both heavier and earlier than in 1946. It will be seen that, in both seasons, the earliest flowering strains suffered the lowest attacks and that those which were slowest in maturing and therefore exposed to the Pea Moth for the longest period showed the highest attack. It is significant, also, that in 1947 when all the yields were low, the heavy pea moth attack on some of the later and commercial varieties reduced the yield of undamaged seed to below that of some of the earlier and less attacked varieties.

TABLE X.

Incidence of attack on certain Maple and Dun pea strains at the Plant Breeding Institute, Cambridge.

Variety	1947 TRIAL Sown 21st April		Type and origin of strain	1946 TRIAL Sown 13th March	
	Date of 1st flowers	Seed attack %		Date of 1st flowers	Seed attack %
A	2nd June	6.6	Maple, P.B.I. ..	20th May	2.00
B	5th June	13.4	Maple, P.B.I. ..	26th May	2.25
C	5th June	13.1	Maple, P.B.I. ..	31st May	4.00
D	6th June	13.4	Dun, P.B.I. ..	29th May	5.40
E	6th June	17.3	Dun, P.B.I. ..	29th May	6.35
F	6th June	15.7	Dun, P.B.I. ..	29th May	6.50
G	16th June	16.1	Maple, P.B.I. ..	6th June	10.50
H	20th June	28.2	Dun, Commercial ..	17th June	15.05
I	26th June	28.3	Maple, Commercial ..	25th June	15.40

Conclusions.

The trials have demonstrated that differences in attack by the Pea Moth occur between the six varieties of peas chosen for the experiment. These differences appear to be due to real variations in susceptibility and the two factors, index of exposure and plant cover probably account for the whole of these differences. It has been possible to measure these two factors, but since they are closely dependent on one another, it has not been possible to give a definite measure of their relative importance other than to say that they are almost equal in this respect.

If larval density is regarded as a direct measure of susceptibility then varieties Kelvedon Wonder and Foremost are less susceptible to attack than the remainder which do not differ appreciably from each other. From the commercial point of view, however, the percentage pod attack is probably a more important measure of the infestation than is larval density. According to this criterion varieties Kelvedon Wonder and Foremost showed the lowest susceptibility and varieties Lincoln Blue and Gladstone the highest; intermediate between these were Onward and Harrison's Glory.

This investigation has shown that by the selection of early maturing varieties with small growth habit the plant breeder may be well able to decrease considerably the incidence of pea moth attack and therefore reduce, or eliminate expenditure on other methods of control.

Of more immediate practical importance the trials have also shown that differences in attack occur between varieties now being grown for the same commercial purpose; thus Onward is more susceptible than Kelvedon Wonder, Lincoln Blue more susceptible than Harrison's Glory whilst little difference exists between Kelvedon Wonder and Foremost.

Summary.

The variations in susceptibility of different varieties of peas to attack by the Pea Moth was investigated and an attempt made to determine and measure the factors concerned. Six varieties of peas differing widely in haulm length and earliness of maturation were used in each of two trials. In the first trial (sown 29th March) the early maturing varieties came into flower before the moths were recorded on the crops and suffered the lowest attacks. The later varieties were exposed to attack over a much longer period and suffered the heaviest infestations. In the second trial (sown 3rd May) the attack was more uniform over all varieties with the early varieties more heavily affected than in the first trial; they were exposed to attack from the beginning of flowering until harvesting.

An estimate of the changes in the active moth population during the flight period was obtained and the varieties were compared in relation to the proportion of this population to which each had been exposed. There was a strong positive correlation between the degree of exposure and the incidence of attack on the different varieties.

The infestation of the varieties was also found to be influenced by the amount of cover which each provided; those with the most dense cover suffered the heaviest attacks.

Statistical analyses showed that the two factors, exposure and plant cover, were closely associated and exerted a joint influence on subsequent attack.

Data from other trials corroborated these findings and showed that strains of peas bred to mature early suffered substantially lower pea moth attack than did the later maturing types from which these had been bred.

Acknowledgements.

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References.

- HANSON, A. J. & WEBSTER, R. L. (1936). The Pea Moth *Laspeyresia nigricana* Steph.—Bull. Wash. agric. Exp. Sta. no. 327, 22 pp.
- NICOLAISEN, W. (1928). Der Erbsenwickler, *Grapholitha* (*Cydia*, *Laspeyresia*) sp., sein Schaden und seine Bekämpfung unter besonderer Berücksichtigung der Anfälligkeit verschiedener Erbsensorten. — Kühn-Archiv, 19, pp. 196–256.
- WRIGHT, D. W. & ASHBY, D. G. (1946). Bionomics of the Carrot Fly (*Psila rosae* F.) I. The infestation and sampling of carrot crops.—Ann. appl. Biol., 33, pp. 69–77.
- WRIGHT, D. W. & GEERING, Q. A. (1948). The biology and control of the Pea Moth, *Laspeyresia nigricana*, Steph.—Bull. ent. Res., 39, pp. 57–84.
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THE CONGO FLOOR MAGGOT, *AUCHMEROMYIA LUTEOLA* (F.), IN A LABORATORY CULTURE.

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(Based on work approved for the Degree of
 Master of Science in the University of London.)

(PLATE XVIII.)

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Auchmeromyia luteola (F.), a Calliphorid fly of a yellow-brown colour, is a familiar object in houses and villages in the warmer parts of Africa, where it rests on walls and ceilings and visits fermenting fruits and liquids. Its larva, the bloodsucking Congo Floor Maggot, is even better known to the African layman. It lives in sandy or dusty floors as an obligatory, but intermittent, ectoparasite of man. It is probably entirely specific to man and is the only such parasite among the Diptera. Roubaud (1913) observed that even where animals and man slept in the same room, the maggots, which cannot penetrate fur, were only to be found where human beings had lain. Occasionally the fly has been seen around the burrows of the wart hog, the host of other species of this genus. The single record by Schwetz (1914) of the larva of *A. luteola* in a wart-hog burrow awaits confirmation.

No case is known of the transmission of disease by the floor maggot. The host feels a slight prick when bitten, and again when the maggot releases its hold. The wound sometimes bleeds and the feeding of many larvae on one part of the body naturally results in some soreness. Beyond this, the writer has experienced no reaction during the two-and-a-half years he has reared them. Certain colleagues, however, who have acted as host for a few days have complained of the pain, or of swelling and irritation afterwards. Wellman (1906) found the bite severe, bringing up weals with burning and itching for four days. Sometimes the larva is a serious nuisance in nature (Gaschen, 1945; Nash, in litt.), and African boys have been known to sit up all night to avoid it.

No record is known of any natural enemy preying upon or parasitising *A. luteola*.

Ectoparasitism on vertebrates is rare among the legless larvae of the higher Diptera. The known instances (besides the five species of *Auchmeromyia*) include certain maggots of the genera *Phormia*, *Passeromyia* and *Neottiophilum*, all found on young birds in the nest (Roubaud, 1915). The pharyngeal structure is modified for sucking blood (Keilin, 1924). Outwardly, however, a maggot appears ill adapted to live as an ectoparasite and, instead of a tubular sucking proboscis with piercing

stylets, it has a pair of rather blunt mouth hooks. The body-form is such as to minimise the chance of reaching the host, of maintaining contact long enough to feed, and of gripping the skin firmly enough to pierce the tissues. *Auchmeromyia* lacks even the sucker-like prothorax possessed by the maggots which live upon birds.

A. luteola has to contend with different ecological and physiological problems from those of the few other maggots that suck blood, and it is of physiological interest by reason of its exceptional resistance to starvation and desiccation. It has developed this capacity probably to a greater degree than any other larva of the Diptera Schizophora.

There are rather few wingless ectoparasites that live away from their host between meals and these must consequently find it "on foot" as many as six times (and preferably 20 times) in order to complete development. The bed-bugs seem to offer the closest parallel to *Auchmeromyia* in their way of life, and it is interesting that the physiological adaptations of these two unrelated groups are somewhat similar. *Cimex rotundatus* Sign. may be almost the only household parasite as widely distributed in Africa (see Mellanby, 1935) as *Auchmeromyia luteola*.

The species was suspected as a possible vector of human trypanosomiasis in the decade 1904-1914. It was then widely observed and studied in Africa by Dutton, Todd & Christy (1904), Wellman (1906) and Rodhain & Bequaert (1913). The monograph by Roubaud (1913) includes long accounts of the biology of *Auchmeromyia* species, especially of *A. luteola*. This worker collected and reared larvae at many places, and tested their tolerance of various climates. He also reviewed and discussed the geographical range.

A. luteola was given little attention by entomologists after it was no longer suspected of carrying disease. Patton (1935) examined the genitalia and erected a new sub-family, the AUCHMEROZYINAE, for the five species of the genus.

It was decided in 1946 to try to cultivate the species in the laboratory in view of its interesting larva. It was felt that a culture would offer useful material for later work on the comparative physiology of blood-sucking insects. Living maggots were received in London from the following places and scientists:—Kota Kota, Nyasaland (*Rangeley*); Fort Johnston, Nyasaland (*Lamborn*); Shinyanga, Tanganyika (*Potts*); Leopoldville, Belgian Congo (*Henrard*); Malakal, Anglo-Egyptian Sudan (*Lewis*); and Hargeisa, British Somaliland (*Anderson*).

Cultivation proved easy and the method used will be described as well as the life history in the culture. The specimens were subjected to various artificial climates, their reactions to which are, of course, only a tentative guide to their biology in nature. It is hoped that field studies may be made in due course in Africa, using modern techniques. The present paper also includes a review of the known geographical distribution and an account of experiments to determine the resistance of the larva to heat and starvation.

Geographical Distribution.

Information under this head has been collected for over two years. The many records published between 1900 and 1920 were compared with the labels in the collections of the British Museum, the London School of Hygiene and Tropical Medicine, the Liverpool School of Tropical Medicine and the Musée Nationale in Paris. I am obliged to the authorities of these institutions for their help.

It used to be the practice to assume a wide and continuous distribution on the basis of scattered records and hearsay reports. Roubaud (1913) showed a range for this species covering the whole of continental Africa south of a line drawn through the southern Sahara. The more restricted range which it has been possible to confirm is shown on the map (fig. 1), on which Roubaud's line is included for comparison. Only two localities—Air in the Sahara, and the Cape Verde Islands—are added, while very large areas have been excluded for lack of reliable records. The fly is pre-

sumably common in some of these; for instance, West Africa south of the Niger, Central Africa north and south of the Congo River, Abyssinia and Somaliland, and Portuguese East Africa. Roubaud's line may be near the correct northern limit, though Lewis (1949) points out that "many insects in the Sudan show great discontinuity in their distribution, which appears to have been caused by past changes in climate."

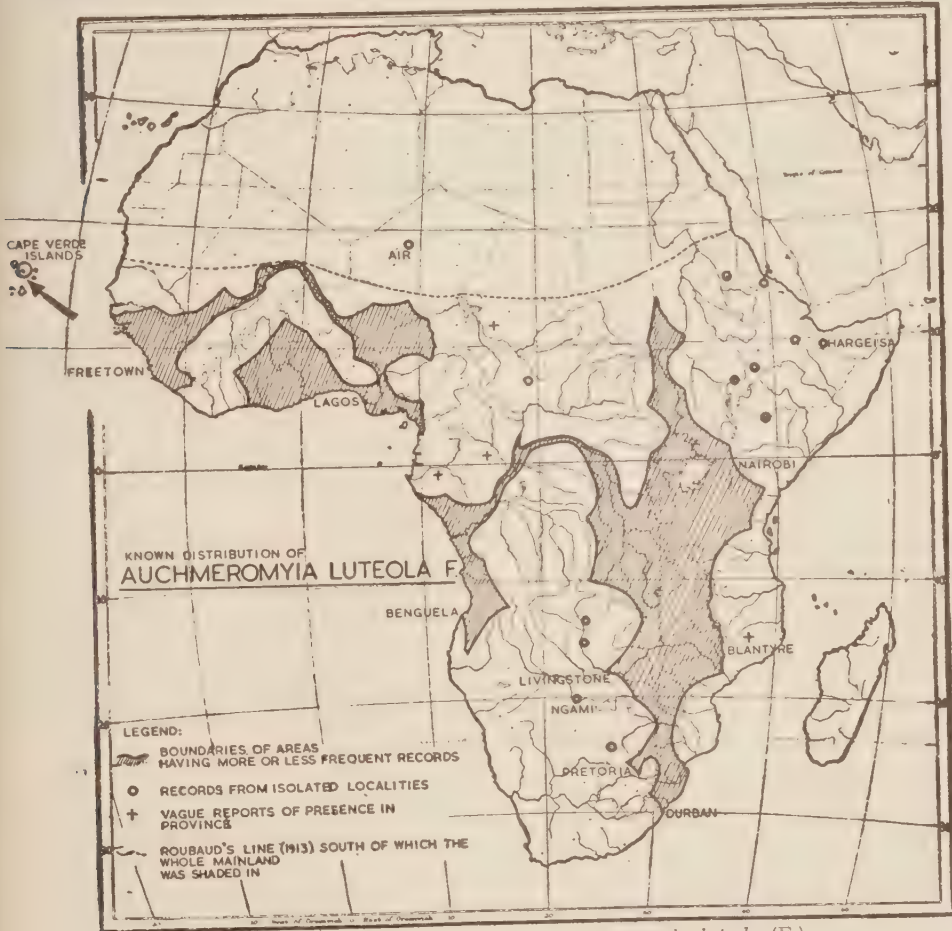


Fig. 1.—Known distribution of *Auchmeromyia luteola* (F.).

The supposition (followed by later authors including James, 1947) that *A. luteola* extends to the Cape is incorrect. A line from southern Angola through Ngamiland to Durban would probably represent its actual limit both now and fifty years ago.

In the Congo and other territories the species has been found "universally", "abundant and generally distributed in all villages", by different authors. This seems to be the rule where collectors have kept a lookout for the maggot or the fly. Records of casual occurrence are few, more often the species is overlooked. In several areas, the medical authorities have been surprised to find it plentiful in their villages when asked to look specially for it.

A. luteola probably exists in all the settlements in areas where the people sleep

without raised beds, and it has, in that sense, a continuous distribution. Nevertheless, if the villages are widely spaced, the parasite may be segregated into populations which cannot interbreed freely. One would expect genetic barriers to have grown up between specimens from different parts of the continent, but flies from Nyasaland were fully fertile if mated with others from Leopoldville. A pair of flies from Leopoldville and Malakal (Anglo-Egyptian Sudan) produced a second generation but failed to produce a third.

The range is remarkable; it includes very wet and very dry macro-climates and is much wider than that of (for example) any species of tsetse fly. Balfour (1909) commented on the wide climatic tolerance of the species, for he met with it in the rain forest and later at Bara (average annual rainfall 10 ins.) in the northern Sudan. Other dry localities are Hargeisa, Timbuctu, the northern part of Air, and the Cape Verde Islands (de Meira & others, 1947). The last is the only insular record to date; Aders (1917) sought it without success in Zanzibar.

At the other extreme it has been found at Freetown, Lagos, and parts of the Cameroons, where the annual rainfall exceeds 80 inches.

The January or the July isotherms within the range vary between 29.5°C. (85°F.) at San Nicolao, Timbuctu and the lower Zambezi, and 12.5°C. (55°F.) at Pretoria, Gaberones and Lake Ngami. The fly occurs also at high altitudes, e.g. on Ruwenzori at 7,500 feet, on Elgon at 6,200 feet, at Mabingo, 6,000 feet, and on the Dchang Plateau, 6,000 feet.

A. luteola is not confined to particular races of man but can establish itself in any floors of suitable loose material where people habitually lie within reach of the maggots. The prevalence of raised beds in parts of East Africa may account for the comparative rarity of the species there.

It is not easy to determine where the range is limited by the sleeping habits of man, and to what extent by climatic conditions. Roubaud pointed out that the larva cannot hope to survive among a nomadic community, but the very climatic conditions, which lead man to wander rather than settle down, may be intolerable to the parasite. Its presence in outpost settlements in the Sahara, however, indicates that in the north "host availability" rather than climate limits the range. The same may hold for the Kalahari Desert, whereas towards Durban it is limited more probably by the relatively cooler climate combined with the prevalence of raised beds.

Method of Cultivation.

The larvae were received between October, 1946 and June, 1947, packed in sand or crumpled tissue paper. Most of them survived the journey, even by surface mail, but specimens which arrived as pupae seldom produced adults; they were thought to have died while pupating in transit. Some larvae were found in the outer packing, having squeezed through the small ventilation holes in the container.

Open glass pots containing a little dry sand were used to rear the larvae. They cannot crawl up a vertical glass surface (nor up any steep incline) when the cuticle is dry. Occasionally a larva escaped from the pot with the aid of its own liquid faeces.

Up to 200 small larvae, or 60 large ones, were kept together in a pot 2 to 2½ inches across, with dry sand about half an inch deep. The pots were placed in an incubator at 23°C., or at 29°C. if quicker rearing was desired. The humidity in both incubators was kept within the range 50–60 per cent. R.H.

The puparia were picked out with forceps as they were formed (usually on the surface of the sand), and placed on sand in a petri dish or corked tube. The dishes were transferred direct to the adults' cages, or placed in separate cages to allow regulation of the numbers of male and female flies in each cage.

Most of the experiments on the larva required controlled conditions. These were obtained by placing, in three incubators (23, 28.5 and 31°C.), airtight jars containing certain mixtures of sulphuric acid and water, providing a known, constant atmospheric humidity in each jar. Small batches of larvae were introduced in open dishes, together with a disc of filter paper or a very shallow layer of sand, to keep them quiescent and avoid the possibility of a microclimate being produced different from that of the jar.

The texture of the larvae is dry and tough and they can be handled easily; indeed they are so elastic that it is not easy to rupture them inadvertently. They can be shaken to the surface of the sand, picked up by hand or with forceps, and tipped from one pot to another without injury.

The larvae were normally fed on the writer twice a week for twenty minutes. They were sieved from the sand, placed in shallow glass dishes (diameter $1\frac{1}{2}$ ") and inverted onto the thigh of the seated host. The dishes were held in place by tapes. Using both legs, twelve dishes of larvae could be fed at a time. A laboratory overall was usually thrown over the dishes during the meal, although (contrary to the experience of Roubaud) it was found that most larvae would gorge readily in full daylight.

Larvae not taken off the host almost immediately after gorging became clogged with liquid faeces. This caused them to stick together in masses for some hours. They were thus prevented from burrowing, and possibly some were retarded or killed in this way.

The larvae are best reared at 23°C. or less when fed (for convenience) only twice a week; at higher temperatures many undersized pupae are formed on this feeding schedule, since in nature the maggot seeks a meal on most nights. The size of the flies reared has declined noticeably over a period of more than two years.

The adults were liberated on emergence into twelve-inch wooden cages with sides of muslin and glass and a muslin sleeve. They were provided with a solution of sugar absorbed in cotton wool, fresh or moistened faeces (either of man or of a laboratory monkey on a vegetable diet), and a tray of clean sand partly wetted with human urine and partly covered with a sweaty sock or cloth to give the smell of used bedding.

The cages of adults were kept in an insectary where the climate was not controlled. Temperatures varied normally between 22 and 28°C., the extremes recorded being 16 and 34°C. Relative humidity ranged approximately from 30 to 50 per cent. In hot, sunny weather the cages were draped with damp cloths, and part of each cage was always shaded.

The relation of the flies to climate was studied by keeping pairs in nine-inch cages in incubators or in rooms in which the temperature and relative humidity could be controlled.

The first eggs were laid in April, 1947, and it was usual to get at least one batch of fertile eggs from each female fly. It was important, however, not to overcrowd the flies. The fertility was high in a cage containing 15 females and 5 males, but fell sharply when 18 females and 18 males were caged together. The longevity of the flies, also, was reduced by more than half, many females dying without ovipositing although their ovaries appeared to be fully developed. Some of the strains died out through overcrowding.

The eggs and young larvae were sieved from the breeding trays and kept in dry sand at 23 or 29°C. until it was convenient to feed them. If necessary, they could be left to accumulate for a week at a time without detriment to the culture.

This method of cultivation was the one normally used. The feeding arrangements for maggots and adults were laborious or inconvenient, and better methods could probably be found. Specimens were fed experimentally on other diets, with results which will be given in describing the life-history.

LIFE-HISTORY.

Egg.

The egg of *A. luteola* is about as long as that of the housefly, but nearly twice as broad, tapering at the anterior end and rounded at the posterior. It is cream-coloured and the surface is covered with a fine reticulate pattern, although appearing smooth to the naked eye. Along the anterior third there is a narrow groove, and the strip of egg-shell which closes it is forced outwards at the head at the time of hatching, and peels away as the larva squeezes its way out of the shell. The empty shell is white.

Batches of eggs hatched 36-60 hours after being laid, when kept at 26-28°C. and 50-60 per cent. R.H. If eggs were transferred after one day from 60 to 0 per cent. R.H., the hatching of some was delayed until the fourth day, whereas others transferred to saturated air all hatched by the second day. At 23°C. the development took 3-5 days in 50-60 per cent. R.H., and 3-7 days in 10 per cent. R.H. A similar delaying effect of dry atmosphere has been noticed in many insects (Buxton, 1932).

Sterile eggs were laid sometimes, and it was not always easy to distinguish these from the eggs killed in experiments. Nine out of twenty are believed to have died in 0 per cent. R.H. at 27°C., while the other eleven produced larvae. Batches of eggs were reared without any failures at various humidities from 10 to 100 per cent. A batch of ten also hatched successfully at 35°C. in 60 per cent. R.H., but in a second batch (perhaps younger when exposed) no larva hatched.

Larva.

The larva has been described by Dutton, Todd & Christy (1904), Wellman (1906), Newstead, Dutton & Todd (1907), Rodhain & Bequaert (1913) and Roubaud (1913). It is a waxy, cream-coloured maggot when newly hatched, 1.5-2.0 mm. long and about 0.22 mg. in weight, with five pairs of pointed fleshy processes on the anal segment. The larva becomes visibly thinner and greyer, if unfed for some days after hatching, but remains as active as before.

The gorged larva is bloated and the cuticle is taut. The branching white tracheae form a conspicuous pattern against the gut distended with fresh blood and there is a collection of small clear bubbles with the blood, possibly of air taken in with the meal. The larva seeks to crawl away and burrow as soon as it has gorged. Its movement at this time is aided by defaecation which wets the whole cuticle, and enables it to climb even up a vertical plane of glass.

When placed on sand in the daylight, the larva (fasting or gorged) immediately tries to burrow and to get an inch or more below the surface. It can do this more quickly in fine dust or silt. The newly-hatched larvae are easily missed in sand, and require careful search; they cannot be sieved as they are quick to wriggle through holes no larger than the sandgrains. A larva on a hard surface crawls away from light but this response becomes lost or weakened in the prepupal stage.

According to Roubaud, a quick temperature rise of 3 to 5°C. brings the fasting maggot to the surface, and he thought that the warmth from the host's body would stimulate it to come up in search of food. Such stimulation is known in the allied Tumbu Maggot (*Cordylobia anthropophaga* Grunb.), but Schwetz (1914) has disputed its occurrence in *A. luteola*. Fasting maggots from the culture were placed on the bench in daylight and first the writer's arm, then a hot iron, were brought to within about a centimetre of one side of them. They showed no reaction but continued on their course away from the window. Next, larvae unfed for four days but lying quiescent in sand were warmed by standing upon it a tube of water at 40-50°C. No larva emerged from the sand in daylight, but in a dark incubator at 29°C. a few larvae (not all) came to the surface within ten minutes each time. Warmth may be one of several stimuli to activity in nature. One collector found that the maggots developed periodicity in their movements, always seeking a host about 24 hours after their last meal. They may respond also to vibration.

When alarmed, for instance by shaking, the larvae assume a characteristic bilobed shape (see Plate XVIII, fig. 2), with a constriction at the 5th and 6th segments. In addition, the head is sometimes retracted. This posture is taken by larvae of all ages, although one that has just gorged does not appear to be able to achieve it.

McConnell (1913) and Roubaud (1915) described how the larva feeds. The process has been watched many times in the laboratory. The body is first arched and the host's skin scraped by the mouth-hooks and the minute toothed maxillary plates in front of them. The larva applies the margin of the prothorax to the skin, having obtained a purchase, and seems able to create a partial vacuum within which the mouth is worked rapidly back and forth. This grip enables the body to be raised clear of the host so that the insect appears to stand on its head while feeding (Plate XVIII, fig. 1). During the meal it keeps enlarging the wound, or renewing the flow of blood, by the piston-like action of the mouthparts and this gives the body a slight shaking motion that can be distinctly felt by the host. Now and then, the whole body undergoes peristaltic contraction from front to rear. It gets so heavy towards the end of the meal that it tends to fall back on to the host, and it may be this that eventually forces the larva to release its grip.

The larvae took about 20 minutes to gorge in the laboratory, but newly-hatched specimens often stopped feeding after 10 minutes. Given the opportunity, a meal was taken daily except for a day missed before each moult. No larva could be induced to bite twice in one day unless the first feed had been interrupted.

The faeces, a thick brown liquid, smell of ammonia as in other Muscid maggots. They are passed just after gorging and perhaps several times more during the next few hours, after which all excretion seems to stop unless pupation is imminent.

Roubaud found that the larva gorged readily only if shielded from light by a surrounding heap of sand but, in the writer's experience, it has been found capable of biting in full daylight and may not release its hold even if brought into direct sunlight.

The larva has been reared experimentally on laboratory animals and on free blood. Some of a batch, placed on a rabbit's ear, bit and took larger or smaller meals; but the struggles of the host disturbed them and allowed many to escape, and after some days they were transferred to a human diet. A better alternative subject was a guinea-pig, anaesthetised with Nembutal and with part of the belly depilated (since the larva cannot readily penetrate fur). At first, few larvae would gorge, development was retarded and the size of the pupae reduced; the mean weight of six pupae obtained by this method was only 46 mg., compared with 61 mg. in specimens reared on man. But with small improvements in feeding technique, good-sized pupae have been obtained and a strain kept going on the guinea-pig through three generations.

Larvae which had fasted for several days were placed overnight in a tray with a partly shaven, active guinea-pig, but they did not bite the pig, and many crawled away to the sides of the tray. Some larvae, however, gorged when placed for an hour near the shaven belly of an anaesthetised guinea-pig wrapped in a cloth.

It was found possible to get the larvae to feed on free blood, a rather rare faculty in bloodsucking insects. Some newly-hatched larvae were placed on filter paper partly soaked in citrated rabbit's blood and these were incubated at 29°C. The whole larva became enveloped in a film of blood as soon as the cuticle, which is hydrophilic, came in contact with it. Although all the larvae imbibed some blood, few gorged. The batch began to die after the second such meal, possibly from clogging of the spiracles with blood. There was only one survivor by the twelfth day, and this continued to feed on free blood until the 23rd day when the test was abandoned.

Other larvae, fed on man until the second moult, were transferred to diets of citrated or defibrinated rabbit's blood. On the citrated blood, five out of twelve were still feeding on the 14th day, and two pupated and produced adults. On the defibrinated blood, three died but three others pupated after five meals of this blood

and one of citrated. Similar results were obtained using oxalated horse blood and heparinised human blood.

No attempt was made to maintain a strain of *A. luteola* through successive generations on free blood.

Pal (1950) found that the floor maggot is one of the very few insects with a cuticle that is both lipophilic and hydrophilic. A liquid, or the liquid excreta of the larva, spreads as a film over the whole larva on contact.

Fasting larvae kept in a saturated atmosphere never gained in weight, so it appears that they neither imbibed moisture nor absorbed any through the cuticle. Indeed, the high resistance to desiccation indicates that the larva can somehow maintain the integrity of the wax layer against abrasion by sand. According to Wigglesworth (1946), abrasion by fine inert dusts can lead to death by desiccation in other insects, while soil-living larvae are permeable to water as a result of natural abrasion.

The cast skins are left on the sand after the larvae have burrowed below. Sometimes a moulted skin is carried on the anal segment for some days where, after the larva has fed two or three times, it is perched like a hat much too small for the wearer. The pharyngeal armature protrudes from the pelt in an inverted position, the mouth-hooks being attached near their apex to the cuticle. Sometimes the main tracheal tubes remain attached to the spiracles, and the foregut to the pharynx.

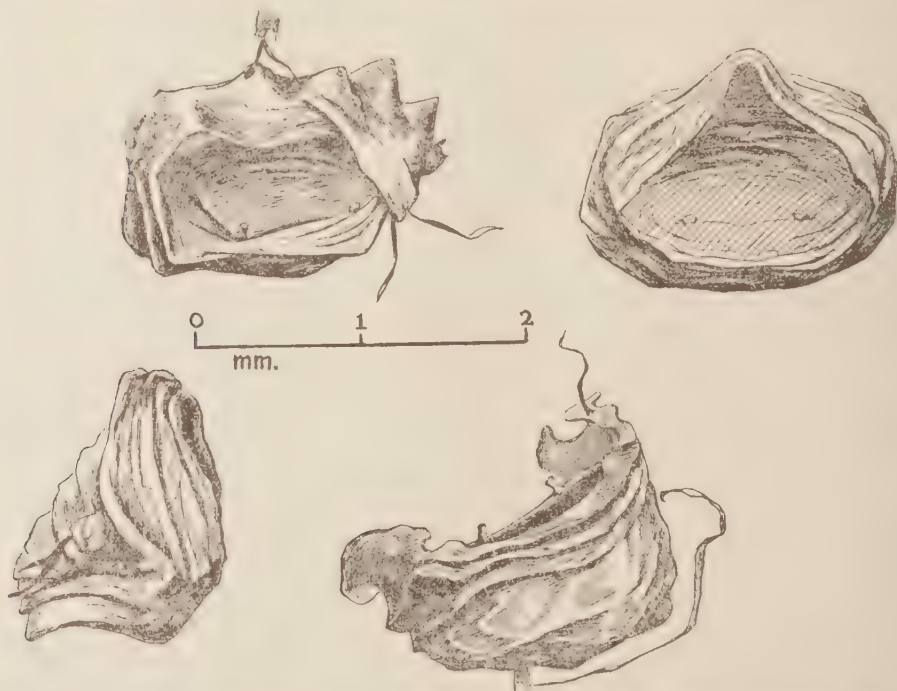


Fig. 2.—Skins cast by larva of *Auchmeromyia luteola* (F.) at second moult: upper sketches, the internal (anterior) and external aspects; lower sketches, lateral and dorsal aspects.

The cast skins (fig. 2) are much more substantial than in most of the higher Diptera, the larvae of which live in moist places. When freshly shed, they are light or dark brown, pliable and rather tough. The second-instar skin weighs about a third of a milligram, a few hours after it is cast, when the larva weighs 12–19 mg. It would probably be easy to detect the presence of Floor Maggots in the field by the skins contained in a sample of the surface sand.

The influence of certain temperatures, atmospheric humidities and feeding schedules, on the growth, development and survival of the floor maggot was investigated. The various responses will be described in the following order:—rate of growth; size of meals and loss of weight between meals; total gain and loss of weight; number of meals and rate of development; weight of full-grown larva and of newly-formed puparium; survival; difference in the size of the larvae and pupae of either sex; the thermal death point; and the fasting maggot. One may temporarily ignore the effects of sex, since the numbers of males and females were consistently almost equal.

Rate of growth.

Batches of ten larvae were simultaneously reared at three temperatures (23, 28.5 and 34°C.), in two relative humidities (10 and 90 per cent.) and on two feeding schedules (2 and 4 meals a week). The experiments lasted from the first meal of the larvae to the emergence of the adults.

Table I shows the mean weight of a larva in each batch at weekly intervals from the date of the first meal, when the unfed larvae weighed about 0.22 mg. All these measurements were taken immediately before feeding the batches.

TABLE I.

Growth of the Floor Maggot on two different feeding schedules in various climates.

Batch	Meals per week	R.H. per cent.	Temp. °C.	Mean weight (milligrams) of fasting larva at end of week—							Mean weight of pupa, 1-24 hr.
				1	2	3	4	5	6	7	
A	2	10	23.0	0.90	—	—	—	—	—	—	—
B	2	10	28.5	1.50	6.0	—	—	—	—	—	—
C	2	10	34.0	1.80	—	—	—	—	—	—	—
D	2	90	23.0	1.14	4.3	12.8	31.0	72.1	90.0	—	55.5
E	2	90	28.5	1.33	6.4	23.0	45.0	94.5	—	—	52.8
F	2	90	34.0	1.11	5.8	14.0	15.8	28.3	35.0	52.0	42.0
G	4	10	23.0	3.53	13.3	54.5	81.5	113	—	—	68.0
H	4	10	28.5	3.33	25.3	90.5	—	—	—	—	50.0
J	4	10	34.0	4.50	13.0	—	—	—	—	—	—
K	4	90	23.0	4.11	14.7	61.4	88.8	118	—	—	66.1
L	4	90	28.5	4.67	36.5	109	113	—	—	—	65.2
M	4	90	34.0	4.90	43.3	135	—	—	—	—	68.2

Mean weight in batches of 10 larvae, of which 0-9 pupated.

In four of the batches no larva completed its growth. The deaths are attributable to desiccation in batches A and B and to this factor, combined with (or anticipated by) starvation, in batches C and J at 34°C.

The differences in rates of growth are seen most clearly in the figures for the third week. The feeding schedule was the strongest influence, far outweighing that of the experimental climates. The correlation of rate of growth with temperature, and its weaker correlation with relative humidity, are seen in the weights reached by batches G-M after three weeks. Batch F, at 34°C., of the batches fed only twice a week, was the slowest-growing and produced the smallest pupae: the combination of scarce food supply with a metabolism accelerated by warmth was so unfavourable that all the specimens died, including the two which, after retarded growth, managed to pupate. On the other hand, the same climate suited the larvae receiving four meals weekly (batch M).

Growth of individuals given four meals per week also appeared to be retarded by dry atmospheres; after three weeks, batch G larvae weighed less than batch K, and batch H larvae less than batch L.

The larvae gained temporary relief each time they were taken out and fed, and consequently batches A, B, C and F would suffer from the infrequency of their out-ings in room atmosphere.

Size of meals and loss of weight between meals.

The survivors in batch L (of Table I) were weighed before and after meals, which were given on Mondays, Tuesdays, Thursdays and Fridays. The increases were calculated as percentages of the weight when hungry (not of the weight when previously gorged). The mean increase at a meal was 73.0 per cent. after a one-day fast and 156.7 per cent. (compared with 152.9 per cent. in batch E) after a three-day fast.

These rates of increase are not constant, however; they decline (see Table II) as the larva grows and as the ratio of surface area to volume falls. At the same time the percentage loss of weight between meals increases. It will be seen that little or no blood was taken by larvae about to moult, while just after moulting more was taken than after similar fasts at other times. Two other batches of larvae, reared under similar conditions and fed at intervals of 5-7 days, increased their weight by averages of 249 per cent. and 260 per cent. at each meal following these longer fasts. The highest increase observed in a batch was 320 per cent. (the mean for eight larvae which had fasted for six days). Meals of the order of two-and-a-half times their own weight were taken by larvae of all three instars.

The size of the meals in the batches in other climates was not measured.

TABLE II.

Weight of meals and of excreta. Per cent. changes of weight in 8 larvae at 28.5°C. and 90 per cent. R.H., fed at irregular intervals.

Instar	Meal	Time (days) since previous meal	Per cent. of weight of gorged larva lost during interval of—			Per cent. of weight of fasting larva gained following fast of—		
			1 day	2 days	3 days	1 day	2 days	3 days
I	1	—	—	—	—	—	—	240
	2	3	—	—	13.0	—	—	216
	3	1	12.8	—	—	(0)	—	—
II	4	2	—	13.7	—	—	202	—
	5	1	12.1	—	—	94.6	—	—
	6	3	—	—	19.6	—	—	161
	7	1	12.0	—	—	(14.3)	—	—
III	8	2	—	17.2	—	—	173	—
	9	1	15.9	—	—	73.2	—	—
	10	3	—	—	36.4	—	—	93.2
	11	1	16.6	—	—	51.2	—	—
	12	2	—	27.7	—	—	87.3	—
	13	1	20.0	—	—	(26.1)	—	—

Note.—At meals 3, 7 and 13, larvae which were about to moult or change into prepupae did not bite.

Certain effects of different meal schedules are shown in fig. 3. The three batches were reared at 28.5°C. in 90 per cent. R.H., the first batch taking 12-15 meals, the second 11 meals and the third 6-7 meals. The measurements are mean values, so that the weights reached by individual larvae are masked.

THE CONGO FLOOR MAGGOT.

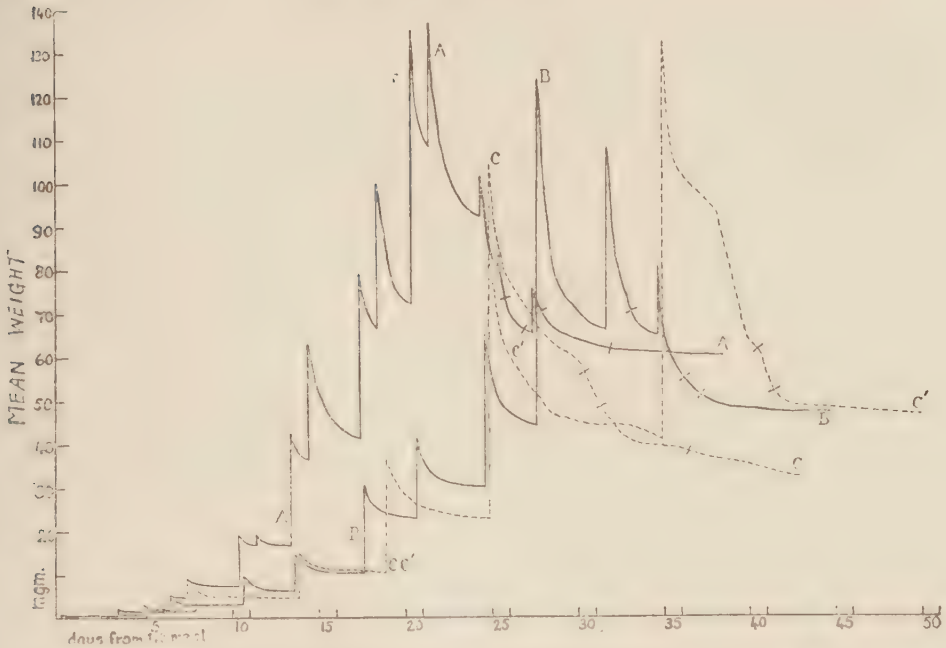


Fig. 3.—Growth of batches of larvae (A, B and C) on three feeding schedules. In batch C some specimens (C') took a seventh meal, the rest pupating after six meals. Pupations shown by cross-lines.

It will be seen that the batch which was fed four times a week, besides growing faster than the others, reached higher maximum and pupal weights and started to pupate on the 26th day. The batches undergoing longer fasts took larger meals, but not of sufficient magnitude to avoid delay in their growth. The delay caused in their development did not correspond, however, to that of their growth, for pupation set in when they were still smaller than the first batch, and lighter pupae and flies were produced. An extended fast after the sixth meal in certain larvae actually induced pupation by the 30th day, whereas in the batch which by then had had nine meals, no larva pupated before the 33rd day. The induced pupation may be a protective reaction against the metabolic risks in a warm climate, coupled with an uncertain food supply. It occurred in the four larvae which at their sixth meal exceeded the mean 97.5 mg. for the batch. The subsequent history of the specimens in this batch is of some interest and will be discussed below (see fig. 5).

Total gain and loss of weight.

Table III shows the overall changes of weight in batches of larvae on different feeding schedules. (The first batch was reared at 27.5°C. and 50–60 per cent. R.H., the others at 23.5°C. and 90 per cent. R.H.) The correlation is evident between the frequency and number of meals and the weight measurements. The growth of the last batch, receiving the fewest possible meals compatible with development in this climate, was striking: the larger meals notwithstanding (139 mg. of blood compared with the 52 mg. taken by a larva in the second batch in its first seven meals), these poorly-fed larvae completed development on less than half the blood taken by the better-fed larvae. They largely compensated for this by much smaller excretory losses, although living in a similar atmosphere. They lost only 23.0 per cent. by weight of their total intake, while larvae receiving about twice as many meals lost 57.4 per cent. Consequently, before and after pupation the worst-fed specimens weighed about three-quarters as much as the better-fed.

TABLE III.
Effect of feeding schedule on size.

Intervals between meals (days)	No. of meals taken	Mean values (in milligrams) of			
		Total blood taken	Total losses between meals	Maximum weight of gorged larva	Weight of pupa at 1-24 hr.
1 and 3	15-17	314	177	137	72.0
1 - 3	12-15	336	193	143	65.2
3 and 4	10-11	302	166	136	52.8
3 - 10	6-7	139	32	107	49.0

Larval gains and losses in other climates were not weighed, but the weight of the pupa (see Table I) is an index of that of the full-grown larva. In a given climate larvae fed four times a week grew larger than those fed twice a week. On four meals a week, the size was similar at 23, 28.5 and 34°C. in high relative humidity, but was reduced at 28.5°C. in low humidity. On only two meals a week, a high temperature resulted in smaller specimens even in the humid atmospheres.

Number of meals and rate of development.

The development of *A. luteola* is easily followed because of the thick skins cast by the larva. The rate of development under various conditions is shown in Table IV, which relates to the same batches of larvae as Table I showing the rate of growth. The period of the whole development within a batch varied by ten days or less.

The time of moulting appeared to be directly related to weight and only through this to the environment. The first moult occurred after the larva had gorged to a weight of 1.5-2.1 mg. (usually at the second or third meal), the second moult after it had reached 12-19 mg. (at the fourth to the seventh meal).

TABLE IV.
Meals taken and rate of development on two different feeding schedules in various climates.

Batch as in Table I	Meals per week	R.H. per cent.	Temp. °C.	No. of meals taken	Mean interval in days, from first meal to—			
					1st moult	2nd moult	pupa-tion	emer-gence
A	2	10	23.0	—	—	—	—	—
B	2	10	28.5	—	10	17	—	—
C	2	10	34.0	—	—	—	—	—
D	2	90	23.0	10-13	9.5	20.5	40.5	57
E	2	90	28.5	9-11	8	16.5	35	45.5
F	2	90	34.0	11-14	8	16.5	40	—
G	4	10	23.0	12	7	18.5	33	50
H	4	10	28.5	10	6	13	27	38
J	4	10	34.0	—	4	13	—	—
K	4	90	23.0	11-14	6	15	33.5	49.5
L	4	90	28.5	11-13	6	13	28.5	39
M	4	90	34.0	6-10	6	11.5	22.5	31.5

Weight of full-grown larva and newly-formed pupa.

There are wide limits of weight, between which the onset of pupation can occur, the upper limit being higher for female than for male larvae. If the feeding schedule (in relation to climate) is favourable, the larvae reach a maximum weight which causes the process to begin regardless of other factors. But if meals are scarce, growth is retarded more than metabolism, and time becomes the limiting factor: the scarcer the meals, the smaller (not the later) the pupae. Finally, the meal schedule may be so adverse that in the time set them by the climate the larvae cannot reach the minimum weight of pupation, and die without pupating. The three possibilities are shown diagrammatically in fig. 4, where curves A, B and C are hypothetical rates of growth determined primarily by the availability of the host. The well-fed larva growing at rate A reaches the weight forcing it to pupate, this being lower for a male larva (A') than for a female. A less well-fed larva will still reach this maximum (at B) if the climate is cool enough to retard metabolism; but, in a warmer climate, the metabolic time-limit will anticipate full-growth and an undersized pupa will be formed after the larva has gorged to the weight represented by the intersection of its growth curve and the time limit. The same will happen to a very poorly-fed larva growing along curve C in the cooler climate, while in the warmer climate it will starve after failing to reach quickly enough a weight which would enable it to form a pupa.

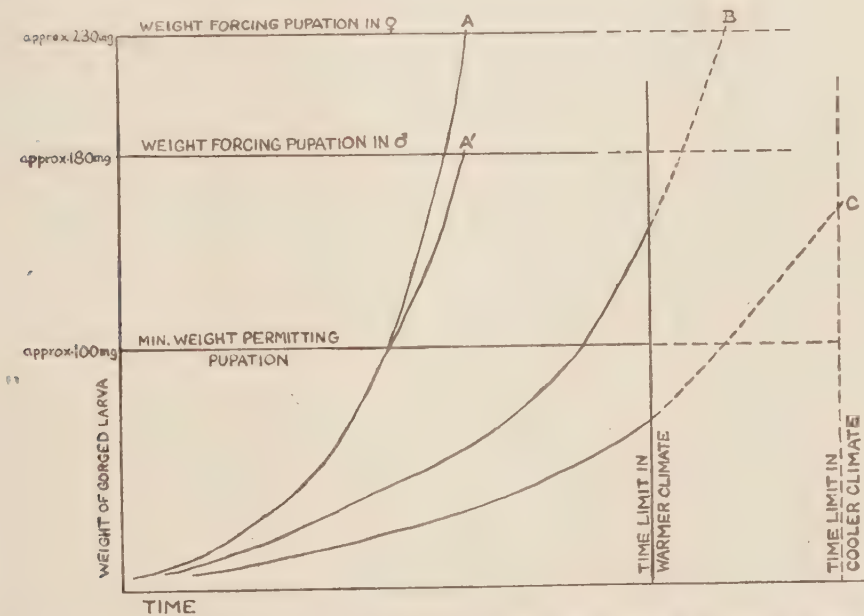


Fig. 4.—Diagram illustrating factors which may determine onset of pupation. Curves A (A'), B and C show growth of gorged larvae at different rates. The point of intersection with one of the limiting factors governs ability to pupate, time of pupation, and size of pupa. The time scale is purely relative.

It is, of course, by no means certain that the weight and time limits do not vary in terms of one another or of other factors in the environment despite the fact that they are shown as straight lines in the diagram. For example, the two curves might join to form a parabola; but this we cannot know on the present data.

The conditions in batches G, K, L and M enabled the larvae to reach the maximum weight for pupation. But where the meal frequency was halved, pupation was brought on by the rate of metabolism before the maximum weight could be reached (see fig. 3). Larvae, fed still less frequently, pupated after six or seven meals on the 29th-41st days, or as soon as those of batch E which received 9-11 meals; in these batches, therefore, the limiting factor was time rather than weight.



Fig. 5.—Pupation (or death) of four poorly-fed larvae of Batch C in Fig. 3.

Certain measurements of individual larvae of this poorly-fed batch are plotted in fig. 5. Weight is lost rapidly in the 24 hours following a meal, but more slowly on subsequent days. The change to the prepupa is marked by a fresh acceleration in loss of weight, which continues rapidly until after the puparium is formed.

The curves show that the prepupal onset occurred earliest in the heavier larvae of the batch; in the case of one of the lighter larvae it occurred just before the seventh meal was offered, with the result that this specimen could neither feed nor finish pupating. The two smallest larvae held out against these conditions long enough to obtain an extra meal, and produced later but larger pupae.

The ability of the larva to pupate when it has achieved only half its normal growth is found also in the mealworm *Tenebrio* (Buxton, 1930). It may be a common defensive faculty in insects occupying warm or dry habitats in which the food supply is erratic.

As few larvae were reared successfully in 10 per cent. R.H., some other batches were kept in rather moister atmospheres, and fed twice a week, to test the effect on the rate of development. At 28.5°C., pupation occurred after about 35 days in 20 per cent. and in 70 per cent. R.H., or at the same rate as in 90 per cent. R.H. On the other hand, a batch at 23°C., in 90 per cent. R.H. took 40.5 days, while one in 60 per cent. took 44 days and another 30 per cent. in 46 days. In the drier climates, net growth would be retarded by evaporation and a possible explanation of these results is that, while at 28.5°C. pupation was determined by the metabolic time limit (see fig. 4), at 23°C. it depended on the weight reached.

No Floor Maggot has been known to complete an instar on a single meal. The minimum for complete development in any climate is probably six meals, two in each stage. Larvae, reared at 28.5°C. in 60 per cent. R.H. on five meals a week, moulted after the 3rd and the 7th meals and pupated after the 16th or 17th. One may suppose that a similar number is commonly taken in nature, and that larvae in cooler floors may gorge more than twenty times if a host is accessible every night.

Survival.

Table V shows the deaths that occurred in the 12 batches indexed A-M. The rates were always high in 10 per cent. R.H., and low in 90 per cent. R.H. when four meals a week were given. The mortality in batches D and E was unexpectedly high, for in other batches reared in the same conditions 90 per cent. of the specimens completed development.

TABLE V.
Death-rate on two different feeding schedules in various climates.

Batch as in Table I	Meals per week	R.H. per cent.	Temp. °C.	No. in batch	Number, which achieved—				Total deaths
					1st moult	2nd moult	pupa-tion	emer-gence	
A	2	10	23.0	10	0	0	0	0	10
B	2	10	28.5	10	1	1	0	0	10
C	2	10	34.0	10	0	0	0	0	10
D	2	90	23.0	10	10	10	9	6	4
E	2	90	28.5	8	7	7	5	5	3
F	2	90	34.0	8	7	6	1	0	8
G	4	10	23.0	10	2	2	2	2	8
H	4	10	28.5	10	3	2	1	1	9
J	4	10	34.0	10	3	2	0	0	10
K	4	90	23.0	10	9	9	8	8	2
L	4	90	28.5	9	9	8	8	8	1
M	4	90	34.0	10	10	10	9	9	1

Only three out of sixty larvae kept at 10 per cent. R.H. pupated and produced adults. Most died when the first moult was due, the rest before and after the second moult. Few larvae died at either moult in 90 per cent. R.H. but rather more died when nearly full-grown. In the batches fed twice a week, there were some deaths among the undersized pupae.

Failure to moult, followed by death, occurred in all the strains in the laboratory. It is believed that this was sometimes due to overcrowding, and sometimes to the larva

being disturbed when ready to moult. The dead specimens turn black and appear somewhat shrivelled but still soft. Inability to moult was commonest in the dry atmospheres and among larvae fed only twice a week.

Saturated air was fatal to the larva but not to the pupa. Larvae were reared with low death rates (5-10 per cent.) at 27-29°C. in air with 90, 80, 70 and 60 per cent. R.H., and two-thirds of a batch survived 20 per cent. R.H. The species tolerates a wider range of atmospheric humidity than most insects (Buxton, 1932), and resembles the bed bugs in this respect. While *A. luteola* can withstand the dry conditions optimal for *Ornithodoros* spp. (with which one would expect to find it frequently associated), unlike these ticks it also flourishes in very moist tropical climates (see Buxton, 1933).

Some third-stage larvae died from what appeared to be rupture of the gut in gorging. Some hours after the meal they had a purplish, bloated appearance and were unable to crawl, burrow or undergo peristalsis. Such individuals became inert and died without developing further. This happened to larvae in batches D, E and K, but in the batches at 34°C. most deaths were presumably thermal. The slow growth combined with rapid metabolism in batch F (see Table I) finally rendered the larvae incapable of gorging or pupating. It seems that the food reserve was used up faster than it could be replaced under these conditions.

Occasionally a larva assumed a bright red colour throughout, and it would retain this through many days of feeding and activity. Such a larva failed, however, to reach full growth or to pupate.

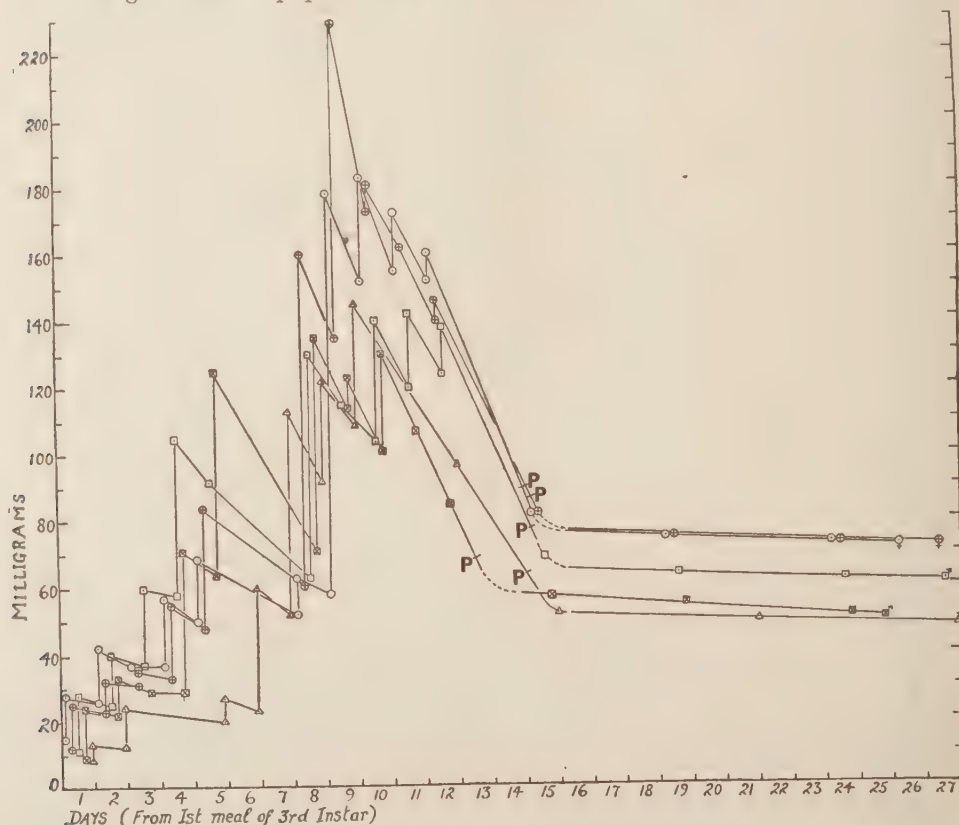


Fig. 6.—Growth and development of two female and three male larvae.

Difference in size of larvae and pupae of either sex.

Female larvae take more blood than males and lose more weight by excretion; they also grow larger and produce heavier pupae. Table VI relates to larvae reared in 1947, when the general size of the specimens was larger than in later tests. They were fed five times a week as seen in fig. 6 where the growth of individual larvae is shown. The rate of development was the same in both sexes.

In all the experiments, female pupae were generally heavier than males of the same batch (Table VII), and the numbers of each were about equal.

TABLE VI.

Difference in size of the sexes, reared at 27.5°C. in 50-60 per cent. R.H.

Specimens	Mean values in milligrams of—			
	Maximum weight of gorged larva	Total blood taken	Total Weight lost from 1st meal to pupation	Weight of pupa 1-24 hr. old
3 males	140.7	245.7	186.6	59.3
2 females	206.5	294.5	212.7	82.0

TABLE VII.

Number and weight of male and female pupae 1-24 hours old, in batches reared under certain controlled conditions (see Table I).

Batch	Meals per week	Temp. °C.	Male pupae		Female pupae	
			No.	Mean weight (mg.)	No.	Mean weight (mg.)
D	2	23.0	3	54.3	3	57.0
E	2	28.5	3	50.0	2	57.0
K	4	23.0	5	64.8	3	68.3
L	4	28.5	4	61.2	4	69.2
M	4	34.0	4	66.2	5	69.8

The thermal death point.

Batches of Floor Maggots were exposed for one hour and six hours to high temperatures combined with various atmospheric humidities, in order to test the influence of humidity on the thermal death point. The apparatus used resembled those described by Beattie (1928), Buxton (1931, a & b) and Mellanby (1932a). A gentle current of air was passed through a series of flasks in a tank of water at constant temperature. The flasks contained sulphuric acid diluted to give the desired

relative humidity, the third one of the series being a wide-mouthed flask (fig. 7) into which the specimens were inserted in a small wire-gauze cage. This permitted quick insertion and extraction without breaking the flow of air or lifting any flask out of the warm water. Insertion caused a drop of half a degree or so in the temperature within the flask, but the temperature regained equilibrium in about five minutes.

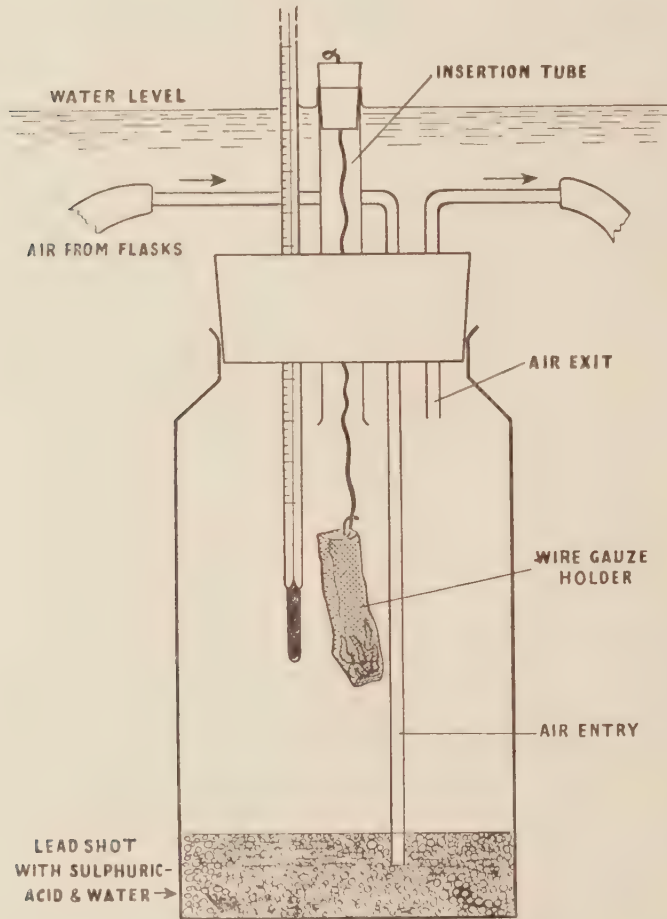


Fig. 7.—Flask in which specimens were exposed for one or six hours to known and controlled temperatures and humidities, to determine the thermal death point.

The results of one-hour exposures are shown in fig. 8. The larvae were exposed, five in a batch, within a few days following the second moult and after a fast of 2-4 days. Nevertheless, differences of size (they weighed 10-50 mg.) and of the state of the gut produced inconsistencies that are small considering the short temperature range.

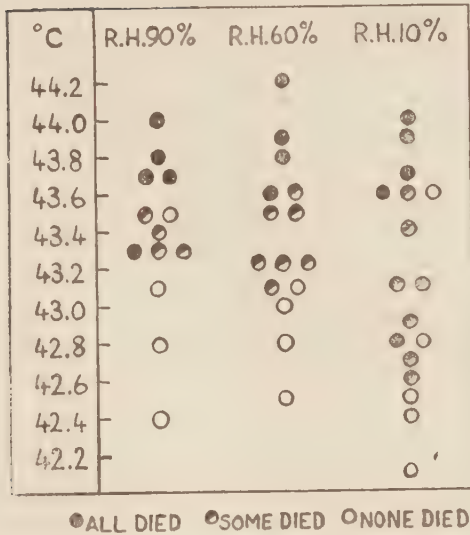


Fig. 8.— Thermal death point experiments on larvae having completed the second moult, at three atmospheric humidities.

A. luteola agrees with certain other insects, such as those tested by Mellanby (1932a), in that the temperature lethal in one hour to a given stage is sharply defined (within about one centigrade degree) and does not appear to vary according to atmospheric humidity. In this respect, Buxton's (1933) classification of physiological types is instructive.

A difference was noticeable in the condition of the larvae just after exposure to dry or to wet heat. Those exposed at 90 per cent. R.H. were soft, blackened and torpid when extracted, responding very slightly (if at all) to touch. They appeared to be dropsical from inability to perspire in the moist atmosphere. Larvae exposed to 10 per cent. R.H. were damp, grey and still active, and most thermal deaths occurred during the 24 hours following exposure. It appeared as if those that survived exposures between 42.5 and 43.8°C. had managed to cool themselves by evaporation during exposure.

A few specimens of other stages were exposed to hot air at 60 per cent. R.H. Some eggs were killed in an hour at 41.6°C. although others survived 42°C. Newly-hatched unfed larvae were killed at 42.5°C. (none were exposed at a lower temperature). All full grown or almost full grown larvae were killed at 43.25°C. and over, but some survived 43.0°C. In the third-stage larva, the thermal death point at this humidity appears to fall slightly with growth. Young pupae may or may not survive exposures at 42.5 and 42.3°C.

Among the surviving larvae, reared under the usual laboratory conditions, there were no long-delayed deaths attributable to the exposure to heat. But, in the exposed pupae, certain deaths occurred about ten days later when the adults tried to emerge.

Some batches of small larvae (weighing 7–40 mg.) were subjected to six-hour heat exposures in air at 60 per cent. R.H. The lowest lethal temperature was 40.4°C. and the highest non-lethal, 41.6°C. It was not possible to select specimens in a uniform alimentary condition, although this is an important factor in long exposures since the accelerated metabolism may cause death by starvation (Mellanby, 1934).

The loss of weight during exposure was measured in certain experiments and the rate of loss in a given climate was found to vary widely. It was generally faster (12.6–17.4 per cent.) at 60 per cent. R.H. in the batches that partly survived a one-hour exposure than in those that wholly succumbed (9.6–10.8 per cent.). These losses were by larvae weighing 10–50 mg. Larger larvae (77–147 mg.) killed by exposure lost only 1.8–7.9 per cent. of their weight. It seems likely that the heated larva opens the spiracles as widely as possible in order to increase evaporation and reduce its temperature, but that these efforts weaken or cease when a certain stage of heat torpor is reached. The proportion of weight lost by evaporation would be lower in the larger larvae whose surface and spiracular area is smaller relative to their volume, and from a given rate of evaporation they would gain less relief than the smaller larvae.

The small larvae killed by six-hour exposures lost 12.3–24.1 per cent. of their weight. As they had already fasted for some days, it seems probable that exhaustion of the food reserves played a part in causing death.

Buxton (1933) recognises four types of reaction to sub-lethal temperatures. The Floor Maggot belongs to the group in which the temperature lethal in one hour was the same at high, medium and low relative humidities. *Cimex* and certain other dry-living insects fall within this group but, as with *Cimex* (Mellanby, 1932b), it is difficult to rear this maggot at any temperature in air with 10 per cent. R.H. or less.

The fasting maggot.

The Floor Maggot can survive fasts perhaps longer than any other Dipterous larva. One kept by Roubaud in dry sand was still alive after 73 days but the same worker found that larvae of various ages kept in damp sand died after 26–33 days.

Larvae from the laboratory culture have been kept unfed in a number of constant climates until they died. Small batches were placed in shallow open dishes in jars with atmospheres of known relative humidity at 28.5°C. and left in a dark incubator undisturbed except at inspection and weighing. Some batches were given a disc of filter paper, others a layer of sand about 2 mm. deep. The larvae were thus well exposed to the desired atmosphere and appeared to remain virtually inactive, an important consideration since much activity would have shortened survival by using up the food reserve more rapidly (Mellanby, 1938).

A larva was considered dead when it failed to show any movement upon shaking or prodding. This usually coincided with discolouration, the larva turning brown in the course of a day or two.

The survival time of larvae in the same batch at 28.5°C. varied greatly (Table VIII). The larvae in 10 per cent. R.H. died of desiccation but continued to show signs of life until shortly before they became quite hard to the touch. Those exposed after two meals were an exception, for they died within two days through failure to moult; larvae left to moult in moist atmosphere, then transferred to dry, lived 2–7 days after moulting (4–9 days after the second meal). The larvae exposed after four meals were not ready for the second moult; those selected for the experiment had failed to moult in the three days prior to exposure. Had specimens about to moult been selected they would probably have been killed sooner in making the attempt.

TABLE VIII.

Effect of atmospheric humidity at 28.5°C. on survival of the fasting larva, and on weight lost before death.

		Stage of development, etc., of the batch at start of experiment.						
		Inst. I unfed	Inst. I after 1 meal	Inst. I after 2 meals	Inst. II after moult	Inst. II after 4 meals	Inst. III after moult	Inst. III at about 90 mg.
90% R.H.	Survival (days)	9-20	8-21	4-21	—	—	17-18	26-47
	Per cent. wt. lost	24.2	44.2	31.8	—	—	37.1	36.1
10% R.H.	Survival (days)	4-9	1-7	0-2	2-7	7-15	—	17-19
	Per cent. wt. lost	62.1	79.2	—	34.5	51.8	—	68.5

Much more weight was lost by desiccated than by starved larvae, while both lost more than larvae killed by one-hour exposures to heat. The figures in Table VIII show the proportion lost from the start of exposure (two or three days after the last meal except in the unfed batches) to the day before the last specimen in the batch died. Loss by excretion is therefore excluded, and the measured losses in both climates were due to evaporation.

Batches of newly-hatched and of third-stage larvae were kept fasting under other conditions. At 23°C. the newly-hatched unfed larva survived 5-37 days in 60 per cent. R.H. and 9-22 days in 10 per cent. R.H., while the third-stage larva survived 25-48 days in 90 per cent. R.H. and 9-19 days in 30 per cent. R.H. The newly-hatched larva lived for 3-8 days at 35°C. in 60 per cent. R.H.

The resistance to fasting shown by the unfed Floor Maggot is very similar to that of the larva of the tropical bed bug, *Cimex rotundatus* (see Mellanby, 1935). It is greater than that of its ally the Tumbu Maggot, *Cordylobia anthropophaga*, of which Blacklock & Thompson (1923) wrote "Left in sand at room temperature, larvae lived without food for about nine days, as a rule; some died much earlier, and a few lived as many as fifteen days." They found that at 37°C. larvae placed on a watch glass lived three days only.

The Tumbu Maggot passes most of its life under the host's (usually furry) skin, in a climate saturated with moisture and above 35°C. The ability of the first instar to resist long fasts in dry places is as important as in all instars of the Floor Maggot, since the appearance of a host must be a matter of uncertainty for both species. Except among endoparasites it is rare to find any insect which can survive 37°C. for a matter of days.

The unfed Floor Maggot, weighing less than a quarter of a milligram, can usually survive for two or three weeks in a climate similar to that of its natural habitat. This is in sharp contrast to most Dipterous larvae which require a constantly saturated environment. A somewhat similar adaptation to drought is found in certain other maggots, e.g. in *Onesia accepta* Mall., a parasite of earthworms in Australia (Fuller, 1933).

Wigglesworth (1946) states that insects from dry environments have harder cuticular waxes than others, so that higher temperatures are needed to melt them and render them permeable to water. This must be true of *A. luteola*, which achieves

exceptional impermeability without the aid of chitinization, although (in the first instar) the ratio of surface area to volume is high. The ease with which both water and fat spread over its cuticle is also worth remarking.

Pupa.

In the laboratory, the puparium is usually formed on the surface of the sand. Roubaud remarks that in Africa the larva takes little trouble to hide for pupation, the pupa lying in loose sand or in a crack in the floor.

The prepupa is an active maggot of a distinctive yellowish colour differing from the off-white of the larva. A few hours before the puparium is formed, the posterior spiracles turn brown. The remainder of the puparium is whitish at formation but soon becomes tinged with pink round the anterior and posterior spiracles and the anus. The retraction of the mouth brings the anterior spiracles to the tip of the puparium, from where the wavy lines of weakness run back across two segments.

A newly-formed white puparium was seen to exude a little colourless fluid from the anus every few seconds, which spread over the cuticle and appeared to evaporate almost at once. This accounts for the continuance of the rapid loss of weight after the puparium is formed.

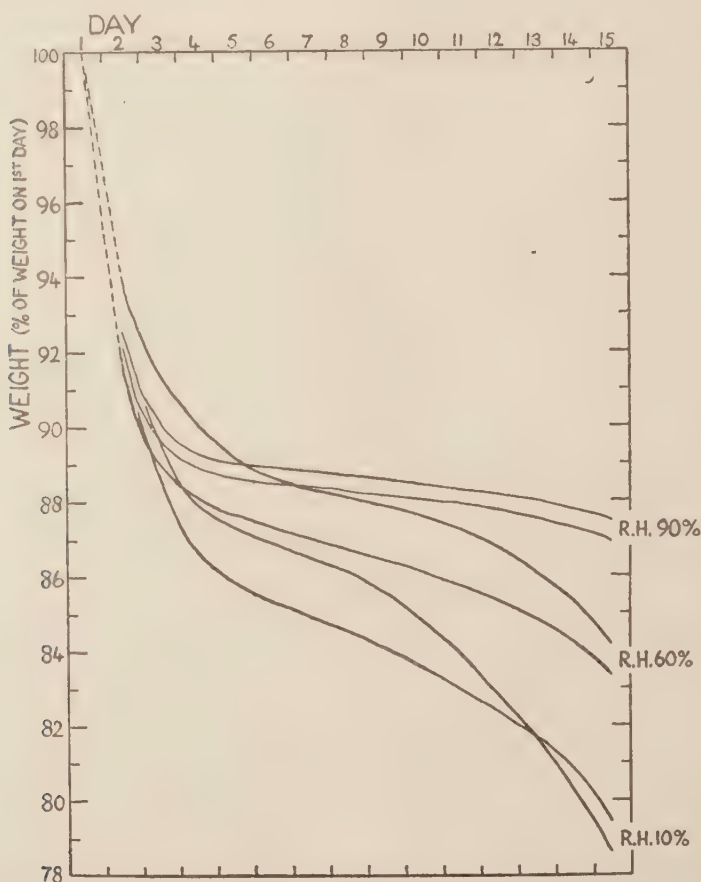


Fig. 9.—Percentage loss of weight in 6 batches of pupae which developed in three different atmospheric humidities at 23°C.

The puparium changes colour to chestnut during the first day, later becoming blackish brown. In both sexes, the pupal stage lasts about 9 days at 34°C., 11 days at 28.5°C. and 15-16 days at 23°C. (see Table IV). It is unrelated to atmospheric humidity.

The larval pharyngeal armature adheres to the inner surface of the puparium when the fly has emerged, while the very flimsy pupal skin is pressed into the posterior end; the well-developed prothoracic spiracles and a hard lump of pink gritty substance are incorporated. In the pupa, the spiracles lie just behind the eye and do not appear to pass through the puparium but to open into a space between it and the pupal skin.

The losses of weight in pupae at 23°C. in three different atmospheric humidities are shown in fig. 9. Altogether 14 males and 15 females emerged, mostly one day after they had been weighed. The first measurement (at the origin of the graph since the percentage losses were based on it) was taken 0-24 hours after the puparia were formed, so that part of the initial loss is excluded. The sigmoid curves indicate three phases. The first lasts (at 23°C.) about 36 hours, when weight is lost very rapidly, chiefly by excretion since humidity hardly affects it. The rate of loss then slackens for about a week. In the final week it accelerates again, but now depends on the evaporative power of the atmosphere. The proportion lost altogether by these puparia was about 16 per cent. in 90 per cent. R.H. and about 25 per cent. in 10 per cent. R.H.

Schwetz (1914) found that pupae were easily killed by slight disturbances. In the writer's experience, it is only during the first few hours that the puparium is really delicate. Older pupae were regularly handled with forceps and may be dropped some inches without injury.

Pupae sometimes died under very dry conditions. Two out of 9 pupae died from exposure to 10 per cent. R.H. at 23°C., while others exposed to 90 and 60 per cent. R.H. all survived. Puparia were formed but no adults emerged when prepupae were exposed to 0 per cent. R.H. at 29°C., but three puparia, less than a day old, under the same conditions produced flies 11 days later, and two of these were able to expand their wings.

The death of a pupa is accompanied by a small but rapid drop in weight. It sometimes occurred early in pupal development, for example, in certain undersized specimens. Not uncommonly, a fly fails to free itself from the puparium and pupal skin and dies in consequence.

An apparent discrepancy in the effects of natural and laboratory conditions has been noticed in several tropical insects, notably in the puparium of tsetse-flies (Buxton & Lewis, 1934). The insect in the soil may surround itself with a layer of moist air, lowering the temperature and the saturation deficit of its immediate environment. This would be the easier for a mobile insect like the floor maggot, which burrows some inches into the floor (Roubaud, 1913) and remains at rest through the heat of the day, metabolising and perspiring economically. Buxton has shown (1936) that in sand, the water-absorbing capacity of which is very small, a water content of less than 1 per cent. may be combined with almost saturated air in the interstices.

The pupa of *A. luteola*, often exposed to room atmosphere throughout its development, remains highly resistant to desiccation. Roubaud (1913) supposed that the species is precluded from attacking desert tribes by the heat in their tents. In this connection the indoor climates in such places as Bara, Air, Timbuctu, the Cape Verde Islands, or Ngami would be worth investigating. Heat, combined with lack of alternative resting places, is more likely to control the winged fly than its early stages.

Adult.

Newstead, Dutton & Todd (1907) refer to preserved puparia in which the position of the fly is reversed, with the head at the posterior pole. Some dozens of dissections have failed to reveal this phenomenon in the laboratory strains, but newly-emerged flies have been seen to jamb their heads into empty puparia.

At emergence the fly violently inflates and deflates the ptilinum, even after the puparium is broken open and although it is lying on top of the sand. It normally seeks a shaded ceiling as soon as it is free, where it settles down to dry its wings.

The sex ratio has remained close to unity throughout in the laboratory, and is unaltered by the environment of the foregoing generation. This does not accord with the experience of Schwetz (1914), who reared 84 males and only 49 females.

Human faeces appear to be a staple diet of the adult. Roubaud found it common near latrines and observed it feeding on fresh faeces on the bank of the Niger. He noticed that the flies were fond also of fallen fruit and of syrup and spirits, and he kept adults for many days on faeces of man, monkey, pig and other omnivorous mammals, but found that those of cattle, dog and cat did not attract them. Wellman (1906) states "On finding that they will eat fermented vegetable matter I was able to keep them under observation for long periods in cages. They are attracted by the smell of vinegar, maize beer or any sour smell, and may be captured by using paper soaked in vinegar as a bait." Graham (1909) reported the adults on a decaying banana and on a palm wine jar.

The results of keeping pairs of flies on various diets are given in Table IX. The guinea-pig meal contained animal protein 5 per cent., dried milk products 10 per cent., by-products of Bemax and products of wheat and maize. The Christopher's Food was a mixture of dog biscuit and Bemax, diluted and fermented.

TABLE IX.

Longevity and fertility of females, each confined with one male, on various diets.

Nature of Diet.								Life in days	No. of batches laid	No. of eggs	No. fertile
Sugar soln.	Human urine	Human sweat	Faeces (man or monkey)	Guinea pig meal	Milk	Christopher's food	Acetic acid 6%				
+	+	+	+	—	—	—	—	53	6	300	275
+	+	+	+	—	—	—	—	54	4	108	98
+	+	+	+	—	—	—	—	24	1	61	?
+	+	+	+	—	—	—	—	57	5	160	149
+	+	+	+	—	—	—	—	43	4	96	35
+	+	+	—	—	—	—	—	14	0	—	—
+	+	+	—	—	—	—	—	17	0	—	—
+	+	—	—	—	—	—	—	15	0	—	—
+	+	+	—	+	+	—	—	15	1	54	29
+	+	+	—	+	+	—	—	> 33*	0	—	—
+	+	+	—	+	+	—	—	> 32*	0	—	—
—	+	+	—	—	—	+	—	25	0	—	—
+	+	+	—	—	—	—	+	12	0	—	—
+	+	+	—	—	—	—	+	9	0	—	—
—	+	+	—	—	—	—	+	7	0	—	—

* Date of death not recorded.

Flies provided with faeces lived about twice as long as those without, although two lived upwards of a month on the guinea-pig meal. This was also the sole alternative diet on which a female developed and laid a batch of eggs, only a rather low proportion of which were fertile. This female died as soon as her eggs were laid.

In nature, the fly spends much time at rest in dark places under eaves, verandahs and so on, but Wellman (1906) saw a number of females in the bush half a mile from any houses, evidently performing a mating flight. He saw several pairs in copula and the flies were still active at dusk. This, with occasional observations of *A. luteola* round wart-hog burrows, may give a clue to the rapid dispersal of the species. Rodhain & Bequaert (1913) found it common in newly-built huts only three weeks after they had set up camp on a long-deserted site. It seems unlikely that the larva is carried about in bed mats, as they supposed.

Several cages, each containing one pair of flies, were kept on the laboratory bench and inspected 36 times over a period of eight weeks. Some pairs were found mated at more than half the inspections, but in subdued light, pairing was reduced to one fifth of the inspections, or less. A newly-emerged male when introduced to a cage did not appear to notice a female as long as she was flying; but after she had settled close to him he approached slowly, then suddenly flew on to her and mated.

Roubaud tells of males attempting to mate with each other, also with dead flies. It is believed that mating always lasts for some hours, possibly for days. If one partner dies, the other sometimes cannot disengage but drags the dead fly about with it.

One male can fertilise several females. Flies from different strains (*e.g.* from Kota Kota, Leopoldville and Malakal) mated as freely as those from the same locality. Pairs were occasionally found in copula in the small closed tubes where they had emerged, before they had fed or had been able to fly freely, although mating usually did not occur for a day or two after emergence.

The female, mated in captivity, often struggles to free herself from the male, usually without success. Either she pushes at him with the hind legs while she makes an angry intermittent buzz (not, it would appear, with the wings), or she shakes her abdomen from side to side in an effort to throw him. One suspects that, in nature, the female spends much time in dark corners where the males cannot easily find her.

Oviposition has been watched several times in the laboratory. When the sand has been partly soiled with urine, some of the eggs are laid attached to the hard edge of sand which has been wetted and since dried. Nevertheless, a tray of dry clean sand was usually preferred to one of urinated sand, especially if it were still damp. It would seem, however, that the smell of human urine in the cage does provide a stimulus to oviposition.

After alighting on the sand, the female might walk a short distance without trailing her abdomen along the surface, but if the conditions were suitable, oviposition started almost immediately. She would suddenly dig the abdomen hard into the sand, extruding the ovipositor as she did so. The hind legs shovelled sand rapidly away to either side while the body was held close to the surface and the abdomen forced downwards as far as it would go. There was a pause of about one second while an egg was laid. The body was then lifted clear of the sand and the hind legs swept sand inwards until the surface was roughly levelled. The fly might next walk a few inches, or might at once lay a second egg almost at the same spot. In either case, she would return later to this spot, with the result that most of the eggs in the batch were grouped in three or four favoured places. These were commonly near or under the edge of the cloth placed on the sand.

An egg was scarcely ever laid in the cloth itself, and no fly was seen to lay one on the surface of the sand, but frequently a few were brought to the surface as later eggs were laid near them. The sand was sometimes slightly furrowed after a fly had oviposited, but more often it was merely pitted from the constant trampling.

The female normally laid about 54 eggs at her first oviposition and this took some 45 minutes at a room temperature of 22–23°C. Several eggs would be laid in rapid succession, then the fly would rest for two or three minutes before starting again.

Patton (1935) was not correct in his view that eggs are laid in a furrow made by the specialized abdomen. The plough-like structure is not used to make a repository for the eggs, though it may be used to find one in a floor where the cracks are hidden by loose sand. Nor is it correct to say that the method differs widely from that of the Tumbu Fly, *Cordylobia anthropophaga*, an account of which, given by Blacklock & Thompson (1923), shows that oviposition is strikingly similar in the two genera, and supports the morphological evidence that they sprang from a common stock.

D. J. Lewis (in litt.) states that in the Sudan the females usually enter tents or huts to oviposit in the early morning. One would expect the smell of such sleeping places to be strongest at that time. Several authors mention that the flies appear to choose ground which has been urinated. In the laboratory, as in nature, oviposition and development continue all the year round without diapause at any season.

As was seen in Table IX, a female has been known to lay six batches of fertile eggs, re-mating between each oviposition. In warm weather, the first batch was laid about 16 days after emergence (or after 20–23 days in flies kept at 23°C.), smaller batches being laid subsequently at intervals of 5–8 days. The longest-lived female and male in the laboratory attained 93 days and 85 days respectively.

Certain females were deprived of male company for various periods. One, isolated from the 26th day, continued to lay eggs some of which were fertile, up to the 54th day. A fly held virgin was incompletely fertilised on the 30th day by the young male then introduced. She laid small batches of eggs on the 59th and 67th days, but only four, in the first batch, produced larvae.

The period of the life-cycle may be roughly estimated. Larvae in an inhabited hut might obtain their first meal three days after the eggs were laid. Finding a host (say) four times a week and living in a floor at 25°C., they would take about another 46 days to develop into flies. The mean interval from emergence to oviposition (allowing for two or three batches of eggs from each female) might be 21 days, giving a life-cycle of ten weeks in all. In these conditions, therefore, *A. luteola* might be expected to complete five generations in a year.

Summary.

An account is given of the life-history of *Auchmeromyia luteola* in a laboratory culture maintained in London for two-and-a-half years. Attention was centred on the bloodsucking larva, known as the Congo Floor Maggot, which is an intermittent ectoparasite specific to man. It was reared chiefly on the natural host, but a strain has been maintained on shorn guinea-pigs through several generations. It was also found possible to rear the larva on free blood.

The known distribution of *A. luteola* is reviewed on the basis of published records, museum collections and information from scientists in Africa. The species is highly successful in both the wettest and the driest parts of the Ethiopian region, but does not seem to extend south of Durban. It can flourish only where man occupies permanent settlements and makes his bed on the floor within reach of the maggot. Strains originating in Nyasaland, in the western part of the Belgian Congo, and in the Anglo-Egyptian Sudan were successfully cross-mated and produced fertile eggs. The second generation from these crosses, however, was not always fully fertile.

The method of cultivation of the material is described.

Batches of eggs hatched 36–60 hours after oviposition when kept at 26–28°C. and 50–60 per cent. R.H. In drier atmosphere development was delayed and took from 3 to 7 days at 23°C. and 10 per cent. R.H.

The habits of the larvae are discussed and it is shown that larvae took 20 minutes to gorge (although newly hatched specimens often stopped feeding after 10 minutes). Given the opportunity, a meal was taken daily except for a day missed before each moult. No larva could be induced to bite twice in one day unless the first feed had been interrupted.

The rate of growth of the larvae was found to be strongly influenced by the feeding schedule, those receiving 4 meals a week having a much higher rate than those receiving only 2. There was also a correlation between rate of growth and temperature but the correlation with relative humidity was less well marked. After fasts of several days, meals of the order of two and a half times their own weight were taken by larvae of all three instars.

Larvae which were fed four times a week and kept at 23°C., besides growing faster than others fed at less frequent intervals, reached higher maximum and pupal weights and started to pupate on the 26th day. Larvae fed at less frequent intervals took larger meals but lighter pupae and flies were produced.

The time of moulting appeared to be directly related to weight and only through this to the environment. The first moult occurred after the larva had gorged to a weight of 1.5–2.1 mg. (usually at the 2nd or 3rd meal), the second moult after it had reached 12–19 mg. (at the 4th to 7th meal).

There are wide limits of weight between which the onset of pupation can occur, the upper limit being higher for female than male larvae. If the feeding schedule (in relation to climate) is favourable, the larvae reach a maximum weight which induces the onset of pupation regardless of other factors. If the meals are scarce, growth is retarded more than metabolism and time becomes the limiting factor; then, the scarcer the meals, the smaller, not the later, the pupae. If the meal schedule is so adverse that the larvae cannot reach the minimum weight for pupation (about 97.5 mg.) in the time set by their metabolic rate in the given climate, death ensues without pupation.

No larva has been known to complete an instar on a single meal. The minimum for complete development in any climate is probably six meals, two in each stage. Larvae reared at 28.5°C. in 60 per cent. R.H. on five meals a week, moulted after the 3rd and 7th meals and pupated after the 16th or 17th.

Failure to moult, followed by death, occurred in all strains in the laboratory. It is believed that this is sometimes due to overcrowding and sometimes to the larva being disturbed when ready to moult. Inability to moult was commonest in the dry atmospheres and among larvae fed only twice a week.

Saturated air was fatal to the larva but not to the pupa. The species tolerates a wider range of atmospheric humidity than most insects, and was even reared successfully in an atmosphere of 10 per cent. R.H.

Female larvae in the third instar take more blood than males and lose more by excretion; they also grow larger and produce heavier pupae and adults.

The temperature lethal in one hour to larvae having completed the second moult is defined within about one degree (42.5–43.5°C.) and does not appear to vary according to atmospheric humidity.

The Floor Maggot can survive fasts perhaps longer than any other Dipterous larva. At 28.5°C. and 90 per cent. R.H. survival of first-instar larvae, unfed, after one meal and after two meals, was 9–20, 8–21 and 4–21 days respectively; third-instar larvae after moult (*i.e.* at 12–19 mg.), and at about 90 mg. survived 17–18 and 28–47 days respectively. At the same temperature but 10 per cent. R.H. much more weight was lost and the survival time was much less. At 23°C. newly hatched unfed larvae survived 5–37 days at 60 per cent. R.H. and 9–22 days at 10 per cent. R.H., while third-stage larvae survived 25–48 days at 90 per cent R.H. and 9–19 days at 30 per cent. R.H. Newly hatched larvae lived for 3–8 days at 35°C. in 60 per cent. R.H.

The pupal stage lasts about 9 days at 34°C., 11 days at 28.5°C. and 15–16 days at 23°C. and is unrelated to atmospheric humidity. Losses in weight at different atmospheric humidities were studied; the proportion lost was about 16 per cent. at 90 per cent. R.H. and about 25 per cent. at 10 per cent. R.H.

The habits of the adult flies are discussed; human faeces appear to be the staple diet. The male seeks the female persistently and mating is protracted and occurs repeatedly. One male can fertilise several females. Oviposition and development continue all the year round without diapause. In the laboratory at 22–23°C. a female normally laid about 54 eggs at her first oviposition, and in one case a female laid as many as 6 batches of fertile eggs. In warm weather, the first batch was laid about 16 days after emergence (or after 20–23 days at 23°C.), smaller batches being laid subsequently at intervals of 5–8 days. The female lived in the laboratory up to 93 days and the male up to 85 days.

The life-cycle under natural conditions is roughly estimated as 10 weeks, so that five generations a year might be expected.

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References.

- ADERS, W. M. (1917). Insects injurious to man and stock in Zanzibar.—Bull. ent. Res., **7**, pp. 391–401.
- BALFOUR, A. (1909). A new locality for the Congo floor maggot.—J. trop. Med. (Hyg.), **12**, p. 47.
- BEATTIE, M. V. F. (1928). Observations of the thermal death points of the blowfly at different relative humidities.—Bull. ent. Res., **18**, pp. 397–403.
- BEZZI, M. (1922). On the Dipterous genera *Passeromyia* and *Ornithomusca*, with notes and bibliography on the non-pupiparous *Myiodaria* parasitic on birds.—Parasitology, **14**, pp. 29–46.
- BLACKLOCK, B. & THOMPSON, M. G. (1923). A study of the Tumbu-fly, *Cordylobia anthropophaga* Grünberg, in Sierra Leone.—Ann. trop. Med. Parasit., **17**, pp. 443–502.
- BUXTON, P. A. (1930). Evaporation from the mealworm (*Tenebrio*: Coleoptera) and atmospheric humidity.—Proc. roy. Soc., (B) **106**, pp. 560–577.
- BUXTON, P. A. (1931a). The thermal death-point of *Rhodnius* (Rhynchota, Heteroptera) under controlled conditions of humidity.—J. exp. Biol., **8**, pp. 275–278.
- BUXTON, P. A. (1931b). The measurement and control of atmospheric humidity in relation to entomological problems.—Bull. ent. Res., **22**, pp. 431–447.
- BUXTON, P. A. (1932). Terrestrial insects and the humidity of the environment.—Biol. Rev., **7**, pp. 275–320.
- BUXTON, P. A. (1933). The effect of climatic conditions upon populations of insects.—Trans. R. Soc. trop. Med. Hyg., **26**, pp. 325–364.

- BUXTON, P. A. (1936). Studies on soils in relation to the biology of *Glossina submorsitans* and *tachinoides* in the north of Nigeria.—Bull. ent. Res., **27**, pp. 281–287.
- BUXTON, P. A. & LEWIS, D. J. (1934). Climate and tsetse flies: laboratory studies upon *Glossina submorsitans* and *tachinoides*.—Philos. Trans., (B) **224**, pp. 175–240.
- DUTTON, J. E., TODD, J. L. & CHRISTY, C. (1904). The Congo floor maggot.—Mem. Lpool. Sch. trop. Med., **13**, pp. 49–54.
- FULLER, M. E. (1933). The life history of *Onesia accepta* Malloch (Diptera Calliphoridae).—Parasitology, **25**, pp. 342–352.
- GASCHEN, H. (1945). Sur un cas d'invasion massive de "vers de case".—Acta trop., **2**, pp. 76–78.
- GRAHAM, W. M. (1909). Report upon entomological observations made in southern and central Ashanti, 1907.—London, Colon. Off.
- JAMES, M. T. (1947). The flies that cause myiasis in man.—Misc. Publ. U.S. Dep. Agric., no. 631, 175 pp.
- KEILIN, D. (1924). On the life history of *Necrotophilum praecustum* (Meigen, 1826) parasitic on birds . . .—Parasitology, **16**, pp. 113–126.
- LARSEN, E. B. (1943). The influence of humidity on life and development of insects. Experiments on flies.—Vidensk. Medd. Dansk. naturh. Foren., **107**, pp. 127–184.
- LEWIS, D. J. (1949). *Glossina tachinoides* in north-east Africa.—Bull. ent. Res., **39**, pp. 529–530.
- MCCONNELL, R. E. (1913). Some observations on the larva of *Auchmeromyia luteola*, F.—Bull. ent. Res., **4**, pp. 29–30.
- DE MEIRA, M. T. V., SERRAS SIMÕES, T. & PINTO NOGUEIRA, J. F. (1947). Observações sobre a fauna entomológica das Ilhas do Sal, Boa Vista e S. Nicolau (Cabo Verde).—An. Inst. Med. trop., Lisbon, **4**, pp. 257–267.
- MELLANBY, K. (1932a). The influence of atmospheric humidity on the thermal death point of a number of insects.—J. exp. Biol., **9**, pp. 222–232.
- MELLANBY, K. (1932b). Effects of temperature and humidity on the metabolism of the fasting bed bug (*Cimex lectularius*), Hemiptera.—Parasitology, **24**, pp. 419–428.
- MELLANBY, K. (1934). The influence of starvation on the thermal death-point in insects.—J. exp. Biol., **11**, pp. 48–53.
- MELLANBY, K. (1935). A comparison of the physiology of the two species of bed-bug which attack man.—Parasitology, **27**, pp. 111–122.
- MELLANBY, K. (1938). Activity and insect survival.—Nature, **141**, p. 554.
- NEWSTEAD, R., DUTTON, J. E. & TODD, J. L. (1907). Insects and other Arthropoda collected in the Congo Free State.—Ann. trop. Med. Parasit., **1**, pp. 3–110.
- PAL, R. (1947). Permeability of insect cuticle.—Nature, **159**, p. 400.
- PAL, R. (1950). The wetting of insect cuticle.—Bull. ent. Res., **41**, pp. 121–139.
- PATTON, W. S. (1935). Studies on the higher Diptera of medical and veterinary importance. . . . The genera *Adichsia* Surcouf and *Auchmeromyia* Brauer and von Bergenstamm (sens. lat.).—Ann. trop. Med. Parasit., **29**, pp. 199–230.
- RODHAIN, J. & BEQUAERT, J. (1913). Nouvelles observations sur *Auchmeromyia luteola*, Fabr. et *Cordylobia anthropophaga* (Grünb.).—Rev. zool. afr., **2**, pp. 145–154.

- ROUBAUD, E. (1911). Les Choeromyies, Diptères nouveaux à larves suceuses du sang des Mammifères.—C.R.Acad. Sci., **153**, pp. 553–555.
- ROUBAUD, E. (1913). Recherches sur les Auchmeromyies, Calliphorines à larves suceuses de sang de l'Afrique tropicale.—Bull. Sci. Fr. Belg., (7) **47**, pp. 105–202.
- ROUBAUD, E. (1915). Les Muscides à larves piqueuses et suceuses de sang.—C.R. Soc. Biol., **78**, pp. 92–97.
- SCHWETZ, J. (1914). Quelques observations préliminaires sur la morphologie et la biologie de la larve, de la nymphe et de l'image de l' *Auchmeromyia luteola*, Fabr.—Ann. trop. Med. Parasit., **8**, pp. 497–507.
- WELLMAN, F. C. (1906). Observations on the bionomics of *Auchmeromyia luteola* Fabr.—Ent. News, **17**, pp. 64–67.
- WIGGLESWORTH, V. B. (1939). The Principles of Insect Physiology. London, Methuen.
- WIGGLESWORTH, V. B. (1946). Water relations of insects.—Experientia, **2**, pp. 210–214.
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FIG. 1. Third-instar Floor Maggot (*Auchmeromyia luteola*) in typical feeding attitude on arm of man.



FIG. 2. Attitude of larvae and prepupae on alarm, when shaken to surface of sand.



THE DISTRIBUTION OF *IXODES RICINUS* (L.) ON THE BODY OF CATTLE AND SHEEP.

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The economic importance of *Ixodes ricinus* (L.), as the vector of louping-ill (MacLeod & Gordon, 1932) and tick-borne fever (MacLeod & Gordon, 1933) of sheep, has stimulated biological studies on this ectoparasite. MacLeod (1932), in Scotland, observed a marked regional distribution of the female tick on this host, the infestation sites being confined to the hairy areas of the head, the bare areas of the axillary and inguinal regions and, to a lesser extent, the regions of short wool around the hairy and bare regions. Milne (1947), in a detailed study of the infestation of hill sheep in Northern England, recorded a similar distribution. In addition, he studied the effect of age, condition and type of fleece on the infestation of individual sheep.

Surprisingly little data are available on the infestation sites on cattle. Milne (1945) refers to heavy tick infestations of cattle in Northern England and states that the attachment sites 'were on all areas of the body except the back and upper sides from the top of the shoulders to the root of the tail.' Edwards & Arthur (1947), in South Wales, found the highest infestations on the hind-quarter region of dairy cattle. These investigators also suggested that there was no significant difference between the degree of infestation of various breeds of cattle. This leaves a considerable gap in our knowledge of the distribution of the parasite on this host, especially for ecological and control work.

In the present studies a considerable amount of data has been obtained on the distribution of female ticks on dairy cattle. The infestation of sheep has also been investigated on one marginal farm in N.W. Cardiganshire.

The Infestation of Cattle.

Technique.

Preliminary observations conducted during the autumn of 1946 showed that, as with sheep, there was a marked regional distribution of the tick on cattle. Irrespective of the degree of infestation it was apparent that the total female tick population on dairy cattle was almost entirely confined to the following regions of the body:

- | | |
|---|---------------------|
| (a) Head: including ears. | (d) Hindlegs. |
| (b) Dewlap: comprising dewlap and neck. | (e) Folds of flank. |
| (c) Forelegs: forelegs and axillae. | (f) Udder. |
| (g) Escutcheon: <i>i.e.</i> the region between vulva and udder. | |

The infestation of the remainder of the body, namely, the belly, sides and back, was extremely light and seldom exceeded 2 per cent. of the total female population at the period of maximum infestation. In the present work a total count refers to the number of females attached on the seven regions listed above.

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The total number of ticks on the various regions was noted separately and, by ear-tagging, the infestation of individual cattle was recorded throughout the period of observation. A total count on heavily infested animals (over 200 ticks per beast) occupied between 20 and 30 minutes: lightly infested animals were examined in about 10 minutes.

The cattle included in this investigation were examined at 7 to 11 day intervals during the seasons of tick activity. No attempt was made to count larval or nymphal stages.

Distribution on various regions of the body.

The distribution of 49,613 females counted on 41 cattle from nine herds in N.W. Cardiganshire during 1947 and 1948 is given in Table I. It is clear that a marked difference occurred in the numbers attached on the seven infestations sites. The most heavily infested region, in all cases, was the forelegs which contributed respectively, for 1947 and 1948, 28.8 and 32.4 per cent. of the total number of ticks counted. The next most favourable positions appear to be the udder and hindlegs, followed in turn by the folds of the flank and the dewlap. The head and escutcheon were lightly infested and together did not contribute more than 6 per cent. of the total population in any one year. The percentage attached on the seven regions was relatively constant for cattle examined in 1947 and 1948. The regional infestation of cattle on a lightly and on a heavily infested farm was also found to be essentially the same (Table II).

TABLE I.

The distribution of *I. ricinus* on seven regions of the body of dairy cattle.

Year	Farms	Total number of female ticks on the following regions of cattle:							Total
		Head	Dewlap	Fore-legs	Hind-legs	Folds of Flank	Udder	Es-cutcheon	
1947	Bryndderwen ..	228	978	2,492	1,682	1,454	1,997	300	9,131
	Cwmere ..	224	1,055	1,858	1,453	1,062	1,048	338	7,038
	Brogynin ..	4	214	758	371	304	404	45	2,100
	Aberleri ..	0	41	261	139	120	198	4	763
	Ynys ..	2	59	257	79	90	102	9	598
	Wernddu ..	0	89	306	169	136	158	15	873
	TOTAL ..	458	2,436	5,932	3,893	3,166	3,907	711	20,503
	% TOTAL ..	2.5	11.7	28.8	19.5	15.4	19.0	3.4	—
1948	Bryndderwen ..	284	1,176	2,385	1,284	1,218	1,760	246	8,353
	Cwmere ..	230	647	2,318	1,542	1,109	1,313	247	7,406
	Brogynin ..	41	102	767	523	322	355	24	2,134
	Aberleri ..	6	25	181	113	83	118	7	533
	Glanclettwr ..	62	166	682	352	309	368	29	1,968
	Penygraig ..	115	420	1,888	954	836	1,265	91	5,569
	Trwyn-y-buarth ..	24	164	1,213	540	430	713	63	3,147
	TOTAL ..	762	2,700	9,434	5,308	4,307	5,892	707	29,110
	% TOTAL ..	2.6	9.3	32.4	18.4	14.8	20.2	2.4	—
1947 and 1948	TOTAL ..	1,220	5,136	15,366	9,201	7,473	9,799	1,418	49,613

TABLE II.

The distribution of female ticks on the body of five dairy cows on a heavily infested (A) and a lightly infested farm (B) in N.W. Cardiganshire. (Based on 7-11 day counts on the same five animals on each farm during April to October, 1948.)

Region of the body	Female tick-infestation of cattle on			
	FARM A		FARM B	
	Total	%	Total	%
Head	230	3.1	62	3.2
Dewlap	647	8.7	166	8.4
Forelegs	2,318	31.3	682	34.7
Hindlegs	1,542	20.8	352	17.8
Folds of Flank ..	1,109	15.0	309	15.7
Udder	1,313	17.7	368	18.7
Escutcheon	247	3.3	29	1.5

The seven infestation sites form well defined regions on the body of the animal and it was possible during the numerous examinations of the cattle to observe the general distribution of ticks on each site. On the head region females were almost entirely confined to the base of the ears and to the area formed by the lower jaw. The lips and nostrils were the feeding sites for the larvae. The nymphs were concentrated on the hairy region immediately behind the nostrils. This zonal distribution of the three stages in the life cycle of the tick was also evident on the foreleg and hindleg region on the animal. In these regions the larvae and nymphs were found below the 'knee' joints (actually the wrist and ankle joints); the larvae being most numerous around the fetlock. The females, however, were attached on the bare or the hairy parts of the upper leg, the axilla and the groin. Females were also evenly distributed on the remaining four attachment sites, but only occasional larvae and nymphs were seen.

Comparison of the infestation of the fore- and hindquarters.

The attachment sites on cattle can be divided into those on the fore- and those on the hindquarters, as follows:—

Forequarters	Head	Hindquarters	Udder.
	Dewlap		Folds of flank.
	Forelegs.		Hindlegs.
			Escutcheon.

The relation between the mean fore- and hindquarter count of female ticks on four cattle at Trwyn-y-buarth farm is shown in fig. 1. On this farm the cattle were exposed to infestation during the whole of the tick season (March to November). In March and April more ticks were attached on the hind- than on the forequarters. During May and June, however, the forequarters carried consistently higher infestations than the hindquarters. This change in the relative infestation of these two regions is also evident from the regression of the percentage of ticks in the forequarters against days after commencement of tick activity in Spring (for 14 D.F., $r = 0.9736$). This percentage increased from 20 to 80 per cent. during the season (fig. 1). It is also apparent that the heavier infestations of the hindquarters during the early stages of activity is balanced by the higher populations on the forequarters later in the season.

The differences observed in the relative infestation of the fore- and hindquarters of the cattle at Trwyn-y-buarth was also evident in three other herds examined during

the spring of 1948 (Table III). The change in the relative infestations of the two regions occurred irrespective of the degree of infestation of the cattle (cf. Bryndderwen and Glanclettwr farms).

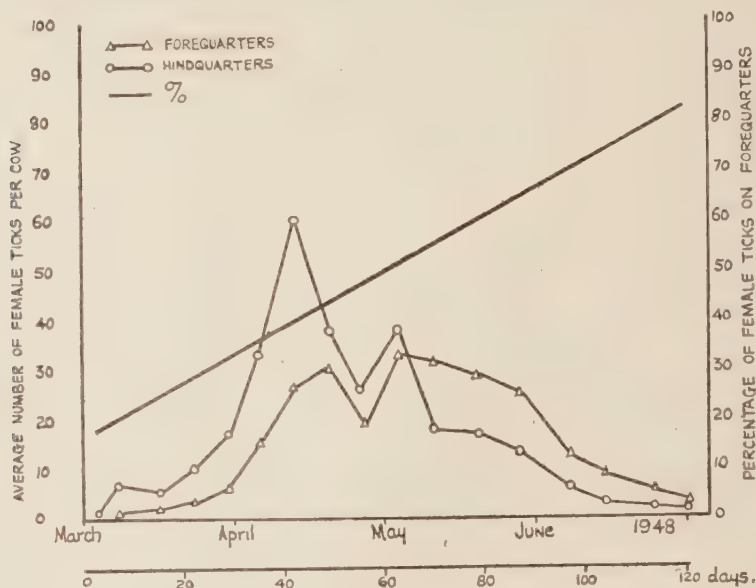


Fig. 1.—The relationship between the mean fore- and hindquarters count of female ticks on four cattle at Trwyn-y-buarth farm and the regression of the percentage of ticks on the forequarters against days after commencement of tick activity in spring.

TABLE III.

The mean fore- and hindquarter count of female ticks on 13 cattle from three herds in N.W. Cardiganshire during April and June, 1948.

Farm	Average No. of female ticks per cow on fore- (F) and hindquarters (H) at 7-11 day intervals during			
	April		June	
	H	F	H	F
Cwmere	6.2	2.2	24.0	31.8
	12.6	7.6	24.8	41.0
	21.8	17.2	14.4	15.2
Bryndderwen ..	80.7	75.7	51.3	44.3
	105.3	73.0	39.3	61.0
	117.7	86.0	23.0	30.7
	125.7	96.3	4.3	9.0
Glanclettwr] ..	6.0	4.2	7.2	10.4
	7.4	6.2	3.6	7.0
	7.0	10.2	2.4	6.0

During the autumn phase of activity the infestations of the fore- and hind-regions of cattle showed a similar trend to that observed in spring (fig. 2). The heavier infestations of the hindquarters at the beginning of activity was again balanced by the heavier infestations of the forequarters later in the season.

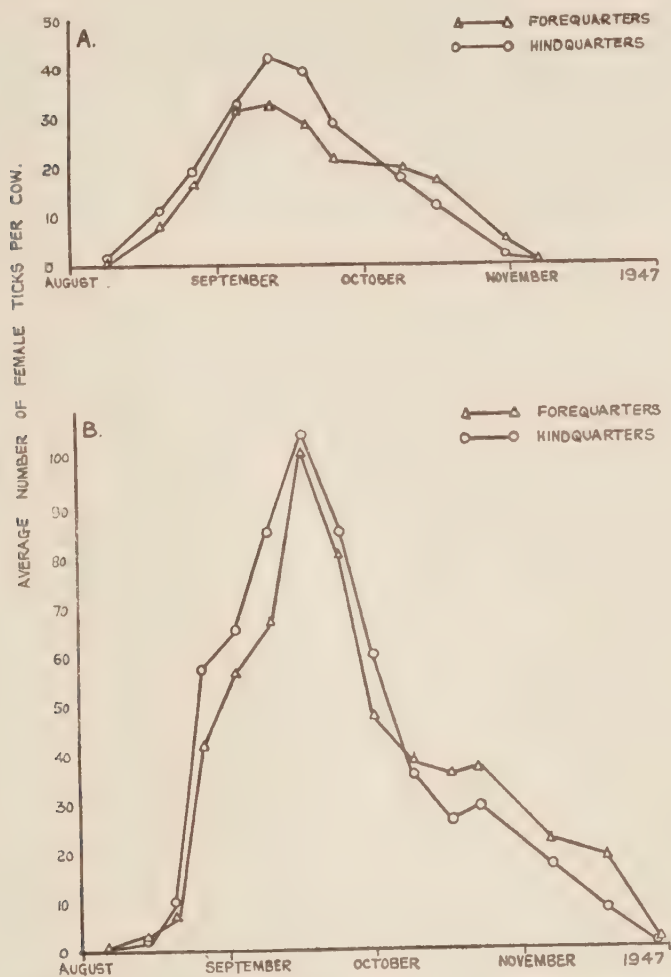


Fig. 2.—The relationship between the mean fore- and hindquarters counts of female ticks on cattle at Brogynin (A) and Brynnderwen (B) farms during August to November, 1947.

This relationship between the fore- and hindquarters was not observed by Edwards & Arthur (1947). These investigators found that the mean number of ticks on the hindquarters was significantly greater than that on the forequarters. It is not possible to discuss the differences between the two series of observations because no indication is given of the periods at which the cattle were examined. It is important to note that marked differences from the relative infestations of the fore- and hindquarters observed in the present study would occur if the majority of the counts was made during the early stages of tick activity, either in spring or in autumn. This would result in a greater number being recorded on the hindquarters and, mask the true relationship between the infestation of the two regions.

Conclusion.

There is a marked difference in the degree of tick infestation of various regions of the body of cattle. These differences have been shown to be relatively constant in

the nine herds examined. The larval, nymphal and female stages show a definite zonal distribution on the head, foreleg and hindleg regions of the body of the animal. Very few larvae and nymphs have been seen on the other regions of the body.

The percentage of females on the forequarters of cattle steadily increases during the course of tick activity, whether in spring or in autumn. During the early part of the season there is a greater number on the hindquarters. Later on in the season the forequarters carry the heavier infestations. The overall effect is for the infestation of the fore- and hindquarters to balance out so that over the entire season there is no significant difference between the number counted in the two regions.

The Infestation of Sheep.

Technique.

The infestation of sheep has been investigated on a marginal farm in N.W. Cardiganshire. For this purpose approximately weekly counts were made of the number of female ticks on the same five ewes and their lambs in the spring and early summer of 1948. The ewes were examined from 27th March to 6th June and the five lambs (all born before 27th March) from 21st April to 6th June. These sheep were restricted to 5 acres of fescue-*Agrostis* pastures invaded by bracken fern (*Pteris aquilina*).

The method of counting the ticks on the sheep in the present studies was the same as that described by Milne (1947). This involved counting the females attached on the head, axillary and inguinal regions of the animal. The axillary region consisted of the axillae and forelegs. The inguinal region comprised the groin, the scrotum or mammary gland, and the hindlegs. The larval and nymphal stages were not counted.

Distribution on various regions of the body of ewes and lambs.

The distribution of 850 female ticks counted on five ewes during the period of observation is given in Table IV. The head region was the most heavily infested

TABLE IV.

The distribution of female ticks on three regions of the body on five ewes.
(The same five Welsh ewes used for each count.)

Date	Number of female ticks on the following regions of the body of five ewes			Total
	Head	Axillary	Inguinal	
1948				
27/3	27	16	13	56
2/4	32	14	17	63
8/4	58	16	16	90
14/4	74	24	19	117
21/4	96	39	28	163
28/4	51	18	21	90
7/5	45	16	18	79
14/5	42	13	6	61
21/5	35	9	4	48
28/5	29	8	6	43
4/6	16	4	1	21
11/6	6	1	0	7
18/6	5	0	0	5
25/6	3	1	0	4
6/7	1	2	0	3
TOTAL	520	181	149	850
% TOTAL	61.2	21.3	17.5	—

and carried 61.2 per cent. of the total number counted on the animals. The axillary and inguinal regions accounted for 21.3 and 17.5 per cent. respectively of the total infestation. This decrease in the number of ticks from the fore- to the hind-regions of the body supports the observations made by Milne (1947) on sheep in Northern England.

The lambs, on the other hand, were more heavily infested on the axillary region (Table V). The head and inguinal regions carried approximately the same percentage of ticks and respectively formed 30.6 and 29.2 per cent. of the total number counted on the five lambs.

TABLE V.

The distribution of female ticks on three regions of the body on five lambs.
(The same five lambs used for each count.)

Date	Number of female ticks on the following regions on the body of five lambs			Total
	Head	Axillary	Inguinal	
1948				
21/4	25	40	31	96
28/4	47	74	53	174
7/5	56	42	30	128
14/5	30	49	40	119
21/5	24	44	46	114
28/5	30	44	25	99
4/6	27	33	11	71
11/6	15	13	10	38
18/6	7	8	3	18
25/6	4	1	3	8
6/7	1	2	2	5
TOTAL	266	350	254	870
% TOTAL	30.6	40.2	29.2	—

The differences observed in the relative infestation of the three attachment sites on ewes and lambs is extremely interesting. Milne (1947) states that this distribution of the females on ewes results 'simply because the sheep walks head first, *i.e.* first come, best served'. If this was the only limiting factor then we would expect a similar gradient in the distribution on lambs. That this was not recorded indicates the presence of some other factor or factors influencing the attachment on sheep. One of the most important factors controlling the movement of the tick on sheep is the extent of the wool covering the body of the animal. Dr. A. D. Lees, Cambridge (see Milne, 1947), has already pointed out that large numbers of females picked on up the fleece of the sheep desiccate before arriving at a suitable site for attachment. It is also possible that the mortality rate of individuals picked up on lambs would not be nearly as heavy as on adult sheep because of the absence of a dense covering of wool, and of the shorter distance the tick would have to travel from the site of pick up to the area of attachment.

An attempt was made in the course of the present investigation to note the effect of the fleece on the distribution of females on the body of sheep. Two shorn and two unshorn wethers were run on the same grazing at Cwmere farm and three weekly counts of the number of females attached on the head, axillary and inguinal regions of the animals was made in May, 1948. During this period 78 parasites were counted on the unshorn sheep as opposed to 113 on those that had been shorn (Table VI). The head region of both groups of sheep carried approximately the same number of ticks, the heavier infestation of the shorn sheep being due to the larger numbers of

females attaching on the axillary and inguinal regions. It appears, therefore, that the fleece of older sheep presents a barrier to the movement of unfed ticks towards the attachment sites formed by the axillary and inguinal regions, and may account for the differences observed between the regional distribution on ewes and lambs.

TABLE VI.

The distribution of female ticks on two unshorn and two shorn wethers run on the same grazing at Cwmere.

Date	Total number of female ticks attached on the following regions of the body of the wethers					
	Unshorn			Shorn		
1948	Head	Axillary	Inguinal	Head	Axillary	Inguin
7/5	21	6	7	26	17	12
14/5	16	6	2	14	8	10
21/5	15	3	2	14	7	5
TOTAL	52	15	11	54	32	27

The infestations of the head regions of ewes and lambs.

Milne (1947) showed that the percentage of female ticks on the head regions of sheep "increase slowly at first and then more rapidly with the advance of the season." A similar increase in the infestation of the head region of ewes has been observed in the present investigation (fig. 3). The increase in the infestation of the head region of lambs is not so evident. The smaller percentage on the head region of the latter may be due to their short covering of wool, which, as has already been shown, permits of a larger number of females to attach on the axillary and inguinal regions.



Fig. 3.—The percentage of female ticks on the head region of five ewes and five lambs during the spring, 1948.

The effect of age on the infestation of sheep.

The mean tick infestation of five ewes and five lambs examined at 7 to 11 day intervals during the spring and early summer of 1948 is shown in fig. 4. An analysis

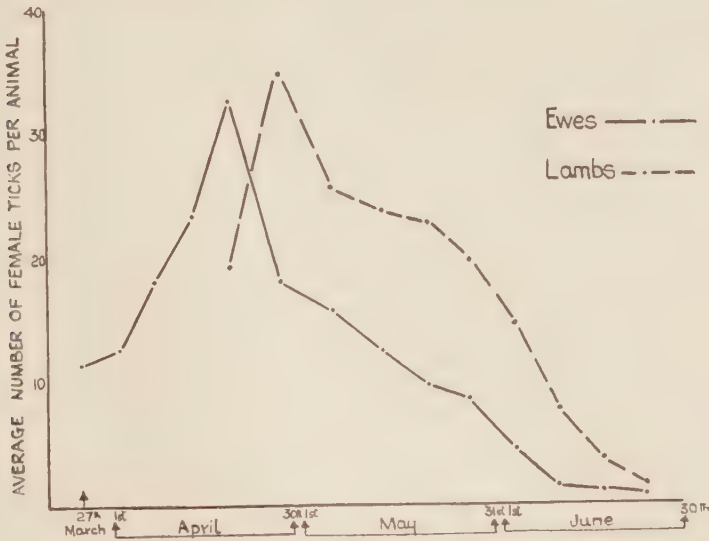


Fig. 4.—The mean tick infestation of five ewes and five lambs on the same grazings at Cwmere farm during March to June, 1948.

of variance of the total number of females counted on the ewes and lambs between the 21st April and 6th June (Table VII) indicated that the lambs carried a highly

TABLE VII.

A comparison of the female tick infestation of five Welsh ewes and five Welsh lambs running on the same grazing at Cwmere 1948.

Animal	No. of female ticks attached on the animal at the following dates										Total
	21/4	28/4	7/5	14/5	21/5	28/5	4/6	11/6	18/6	25/6	
Lamb 1 ..	15	51	27	26	27	19	12	7	2	0	186
2 ..	24	42	32	22	15	17	17	12	3	1	185
3 ..	17	25	26	28	12	24	21	11	8	6	178
4 ..	21	22	17	19	49	26	12	6	1	1	174
5 ..	19	34	26	24	11	13	9	2	4	0	142
Mean ..	19.2	34.8	25.6	23.8	22.8	19.8	14.2	7.6	3.6	1.6	173.0
Ewe 1 ..	32	15	6	15	10	2	7	3	1	0	91
2 ..	31	37	22	14	13	18	6	1	0	1	143
3 ..	34	19	11	11	11	13	1	2	3	2	107
4 ..	37	10	22	9	9	4	2	1	0	0	94
5 ..	29	9	18	12	5	6	5	0	1	1	86
Mean ..	32.6	18.0	15.8	12.2	9.6	8.6	4.2	1.4	1.0	0.8	104.2

significantly greater number than the ewes, but that there was no significant difference between the infestation of individual ewes or lambs.

The degree of infestation of the lambs and the ewes is probably determined by the interaction of two main factors :

(a) the amount of ground covered by the animal, and (b) the nature of the fleece. The amount of ground covered by the sheep is dependent on the distance it travels and the breadth of its body. This factor controls the number of ticks picked up by the animal since, by covering more ground, the sheep picks up more parasites. During the first few weeks after lambing the ewes are more heavily infested than the lambs. This is probably due to the smaller numbers of ticks picked up by the lamb in its restricted wanderings around the mother ewe. As the lamb ages the rapid increase in the size of its body, coupled with a greater degree of activity, narrows the gap between the number picked up by the two age groups. Finally, a stage is reached when the number attaching on the lambs exceeds the number on the ewes. It is probable that this stage is reached fairly early in the life of the lamb since only a percentage of the total number of parasites climbing on the fleece of the ewe succeed in reaching a suitable site for attachment. In the present investigation the lambs were about six weeks old when they recorded heavier infestations than the ewes.

Milne (1947), in Northumberland, recorded heavier infestations of ewes than lambs on the same grazing. This, coupled with a lighter infestation of hogs than ewes, prompted him to suggest that the degree of infestation is directly related to the size of the animal, *i.e.* the larger the animal the greater the degree of infestation. In the flock examined by Milne, however, the lambs were born in Mid-April. If there is a lag of about six weeks before the infestation of the lamb exceeds that of the ewe, then any differences between the infestation of these two age groups would be minimised at the low rates evident in June. Some evidence for this statement is provided by the fact that Milne observed an increase in the infestation of the lambs relative to that of the ewes during the period 28th April to 23rd May, and that in June the infestation of the lambs was heavier than hogs on the same grazing.

Conclusions.

A comparison of the number of female ticks on the head, axillary and inguinal regions of the body of ewes and lambs showed that the infestation of these regions differed in the two age groups. This was due, in the main, to the fleece of the older sheep acting as a barrier to the movement of unfed parasites towards the axillary and inguinal regions.

The percentage of ticks on the head region of ewes and lambs, increases during the course of the spring phase of activity.

Five lambs, born in mid-March, carried a highly significantly greater number of females than their mothers between 21st April and 6th July. Under the prevailing conditions there was no significant difference in the infestation of individual ewes or individual lambs.

The Value of standard Counts in estimating the total Tick Infestation of Cattle and Sheep.

The time and labour involved in counting the number of female ticks attached on the body of the host would be decreased if the total infestation could be estimated from sample counts. Milne (1947) has shown that this is possible for adult sheep and has used the term *standard* count to denote the number of ticks attached on the area chosen for the sample count. This technique has not been tested for cattle or lambs.

(i) Cattle.

The fore- and hindquarter regions of cattle, owing to their clear definition, are probably the most suitable areas for sample counts. A comparison of the number of females attached on these regions in relation to the total infestation of cattle is shown

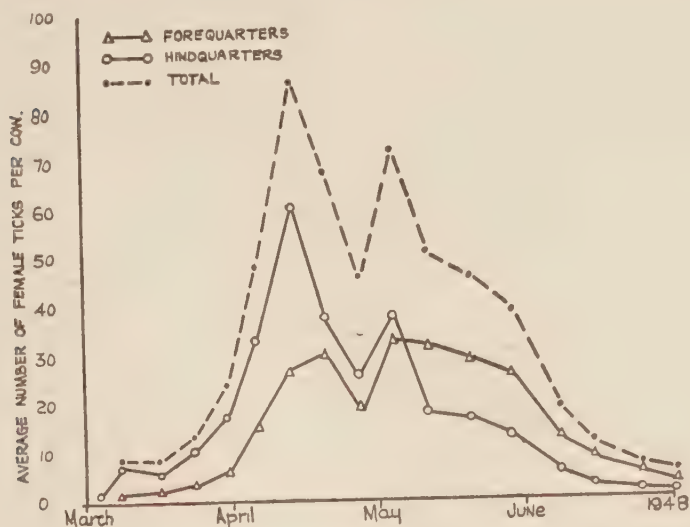


Fig. 5.—The tick infestation of the fore and hindquarters of cattle compared with the total infestation.

in fig. 5. It is evident that the hindquarters count gives a good picture of the seasonal incidence on this host. The forequarter count is not so reliable, and in this instance the peak infestation of the forequarters occurred three weeks later than that obtained from the total tick count.

The relationship between the total and hindquarter count of parasites on cattle at monthly periods during the tick season is given in Table VIII. The data is based

TABLE VIII.

The ratio of the total to the hindquarter count of female ticks on cattle.
(Based on counts on 13 cattle from three herds in 1947 and 1948 and from 13 cattle from three other herds in 1948 only.)

Period	Total (T) and hindquarter (H) counts of female ticks on six herds examined during 1947 to 1948		Ratio of total to hindquarter count	Range between herds
	H.	T.		
March	1,166	1,613	1.383	1.304-1.507
April	4,293	6,677	1.555	1.500-2.047
May	5,512	9,986	1.812	1.696-2.845
June	2,294	4,765	2.077	1.838-3.556
July	445	907	2.038	1.334-3.286
March to July ..	13,710	23,948	1.747	1.621-1.931
August	2,288	3,749	1.639	1.463-1.903
September	8,000	15,396	1.925	1.585-2.025
October	1,741	3,535	2.030	1.580-2.316
November	111	296	2.667	1.500-2.690
August to November ..	12,140	22,976	1.892	1.624-1.994
March to November ..	25,850	46,924	1.815	1.732-1.938

on serial counts on 26 cattle from six herds. The value 1.815 obtained for the ratio of the total to the hindquarters count, during the period of observation, was relatively constant in the six herds (range 1.732 to 1.938). This ratio varies considerably in different months and between herds during the same months, *e.g.* the ratio for April was 1.555 (range 1.500 to 2.047) and for June 2.377 (range 1.838 to 3.556). These variations render this method of estimating the total infestation of cattle extremely unreliable and would be of no value for experimental work.

A significant increase in the percentage of ticks on the forequarters of cattle during the spring and autumn phases of activity has already been noted. The regression coefficient, however, varies from farm to farm (fig. 6). The significant

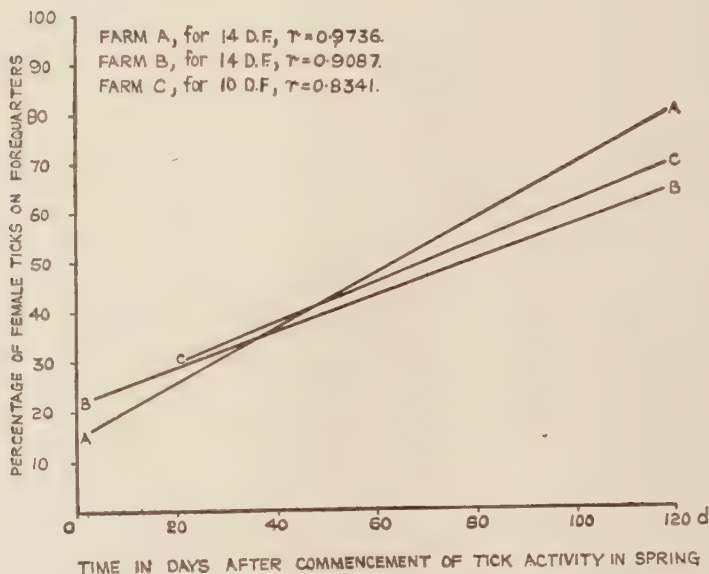


Fig. 6.—The regression of the percentage of female ticks on the forequarters of dairy cattle against days after commencement of tick activity in Spring (March) at Trwyn-y-buarth (A), Bryndderwen (B) and Glanclletwr (C) farms.

difference observed between the regression coefficient for herd A and B does not permit of the use of a joint regression line. In these circumstances the technique is unreliable for estimating total infestation.

(ii) Sheep.

Milne (1947) used the head and axillary regions as standard areas for tick counts on sheep and suggested that the standard count multiplied by 1.185 should approximate the total count at any period during the spring phase of activity. This close relationship between the total and standard count was also observed in the present investigation (fig. 7). During the spring the mean ratio of total to standard count was 1.198 and remained relatively constant throughout the period of observation; for twelve weekly counts the range being 1.141 to 1.300. The relation between the observed and calculated total infestation of the sheep is shown in fig. 7.

The relationship between total and standard count of female ticks on lambs is shown in fig. 8. The mean ratio of total to standard from 21st April to 6th June was

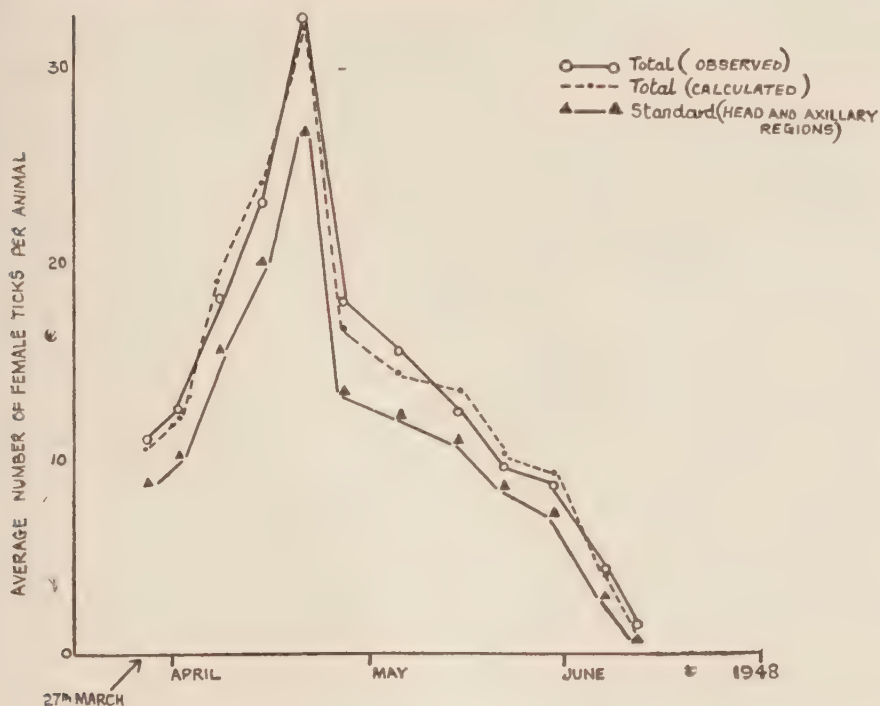


Fig. 7.—The relationship between the mean total and standard counts of female ticks on five ewes and a comparison of the observed and the calculated total count.

1.409 and the range for ten weekly counts 1.183 to 1.691. This ratio is not as reliable as in the case of older sheep. The discrepancy occurs towards the end of a phase of tick activity when the increase in the percentage of ticks in the head region of the lambs has a marked effect on the mean ratio of total to standard count. This method of estimating the total infestation of lambs may be improved by the use of two constants, one for infestations during April and May and the other for the lighter infestations in June. The ratios calculated for these two periods were respectively 1.426 (range 1.340 to 1.691) and 1.238 (range 1.183 to 1.357). The standard count and the appropriate constant then give an estimate of the total infestation (fig. 8).

It is evident that the standard count is also of value in defining the seasonal incidence on both ewes and lambs.

(iii) Conclusions.

The numbers of parasites attached on the hindquarters of cattle when observed at short intervals (7 to 11 days) during the seasons of tick activity, may give a good indication of the seasonal incidence on this host. Neither the forequarter nor the hindquarter count alone is reliable for estimating the total infestation of cattle.

The mean ratio of total to standard count of females on ewes and lambs may be used for estimating their total infestation at any period during the spring phase of activity. It is suggested that the accuracy of the calculated total infestation of lambs can be improved by the use of two ratios, one for April and May and the other for June. The standard count may be used also for defining the seasonal incidence on these animals.

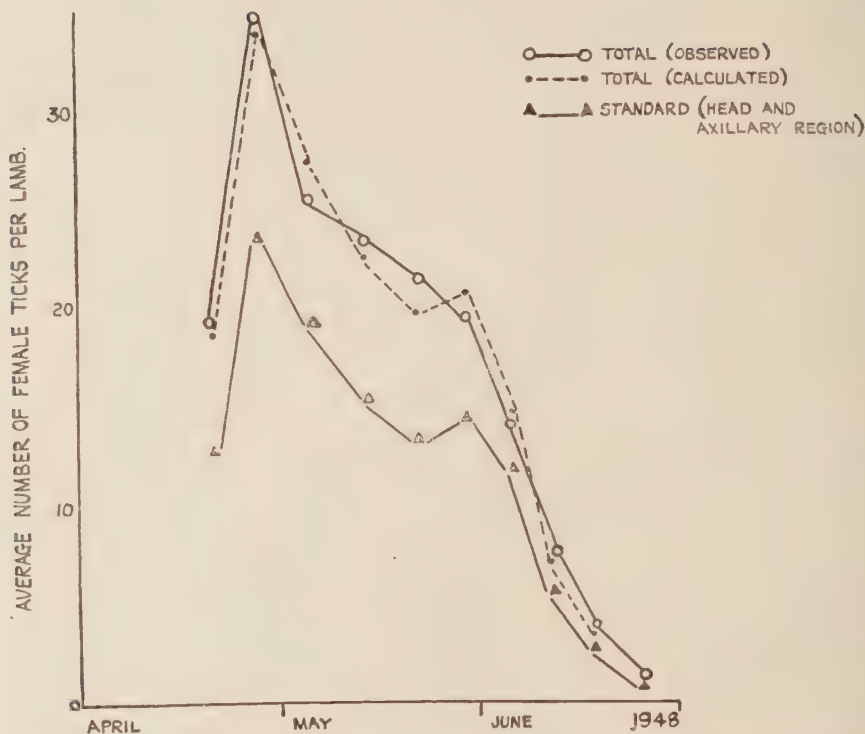


Fig. 8.—The relationship between the mean total and standard counts of female ticks on five lambs and a comparison of the observed and the calculated total count.

Discussion.

The attachment sites of female ticks on cattle and sheep are almost entirely confined to the head, axillary and inguinal regions. The percentage of the total population carried on these regions has been found to be relatively constant between individual animals. The difference between the relative infestation of these regions in ewes and lambs has been shown to be due, to a certain extent, to the barrier formed by the fleece towards the movement of unfed ticks in the direction of the axillary and inguinal regions of ewes. It is possible that the more even distribution on lambs is also due to the smaller size of the body. The difference between the relative infestation of the fore- and hind regions of cattle is extremely interesting. It is generally accepted that the majority of unfed ticks are picked up on the fore region of sheep (Milne, 1947). If the same applies to cattle then the changes observed in the relative infestation of the fore- and hindquarters in spring and autumn must be due to (a) a deliberate movement of ticks towards the hindquarters during the early stages of tick activity, and (b) the attachment of ticks near the site of pick up later in the season. Apart from the above observations there is no evidence for (a) but Lees (1948) has shown that younger ticks (recently moulted) require a more prolonged stimulation for attachment than older and more hungry individuals and, as a result, wander further from the site of pick-up. At the beginning of the "tick season" young ticks will predominate and attachment will occur at a considerable distance from the point at which they climbed on. The higher proportion of older individuals later in the season might result in attachment occurring near the site of pick up. The only alternative is for the pick-up sites to change during the course of activity.

i.e. first the hindquarters and then the forequarters acts as the major pick-up site. These suggestions have been brought forward primarily for future work on this problem rather than as an explanation of the phenomenon.

Summary.

Female ticks are almost entirely confined to head, axillary and inguinal regions of cattle. Larval and nymphal stages attach on the head and below the hock joints on the fore- and hind-legs.

The percentage of females on the forequarters of cattle increases during the course of the season, whether in spring or autumn; during the early stages of a season's activity the hindquarters carry a greater number than the forequarters. Later in the season the position is reversed. Suggestions are put forward to account for this phenomenon.

The head, axillary and inguinal regions are the major attachment sites of the female tick on sheep. In ewes the head region carries the higher percentage, but on lambs the axillary region is the most heavily infested. This difference may be due, to some extent, to the fleece of the ewes forming a barrier to the movement of unfed ticks towards the axillary and inguinal regions.

Lambs carry a heavier infestation between the 21st April and 6th June than ewes on the same grazing. This phenomenon has been discussed in the light of previous work in Northern England.

There is no reliable method of estimating the total infestation on cattle. A good picture of the seasonal incidence of the female tick on the host can be obtained from the hindquarters count. The standard count (the forebody) on sheep may be used for estimating total infestations.

Acknowledgements.

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References.

- EDWARDS, E. E. & ARTHUR, D. R. (1947). *Parasitology*, **38**, pp. 72-85.
 LEES, A. D. (1948). *J. exp. Biol.*, **25**, pp. 145-207.
 MACLEOD, J. (1932). *Parasitology*, **24**, pp. 382-400.
 MACLEOD, J. & GORDON, W. S. (1932). *J. comp. Path.*, **45**, 240-256.
 MACLEOD, J. & GORDON, W. S. (1933). *Parasitology*, **25**, pp. 273-283.
 MILNE, A. (1945). *Ann. appl. Biol.*, **32**, pp. 128-142.
 MILNE, A. (1947). *Parasitology*, **38**, pp. 34-50.

THE ENTOMOLOGY OF SWOLLEN SHOOT OF CACAO.

I.—THE INSECT SPECIES INVOLVED, WITH NOTES ON THEIR BIOLOGY.

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The initial work on the swollen shoot disease of cacao in the Gold Coast (Posnette & Strickland, 1948) made it abundantly clear that critical investigations into the biology and bionomics of the vector species would have to be undertaken.

A preliminary investigation of the Coccid species that occurred on cacao in West Africa was carried out by the writer in 1945-46, and the results have already been published (Strickland, 1947). Two important facts emerged from this work, that many species of Pseudococcids occurred more or less commonly on West African cacao, and that the commonest and most important species (*Pseudococcus njalensis* Laing), was almost invariably attended by ants in the field.

The problem was, in many respects, unique. Investigations covering what Carter has described as "Vector, Virus, and Host Plant . . . an inseparable ecological Trinity" (Carter, 1939) are now more or less commonplace. In the present case, however, the situation was complicated by the addition of some 70 species of ants which were found to be associated to a greater or lesser degree with the 14 known vector species, and hence with the spread of the virus. Again, at the time the investigations were commenced swollen shoot was the only virus known to be carried by members of the superfamily Coccoidea. This introduced another complicating factor, because it was very quickly found that the density of the West African cacao mealybugs was extremely low (roughly 70 per tree). Hence, special techniques had to be evolved for computing vector density in the field.

In a temperate country, with a relatively well known insect fauna, a list of the species involved, with suitable references, would probably suffice as an introduction to the biological and ecological investigations necessary for an adequate understanding of the field transmission of a virus. Unfortunately, in this case, the insect fauna of cacao was virtually unknown and many of the species encountered in the work were either new to science or so little known that accurate determination proved impossible.

Accurate identification is essential when a number of different species has to be dealt with on a quantitative basis. Consequently it was necessary, before embarking on quantitative field studies, to separate out the assemblage of species under investigation into valid species whenever possible, or into groups where specific determination was not possible or was unnecessary as, for instance, in the case of the relatively unimportant Tapinomine ants which are referred to collectively as *Tapinoma* spp.

The present paper details the species involved with such notes on their biology and habits as are pertinent, and forms an introduction to a second paper that is to follow.

TAXONOMIC NOTES.

Coccoidea.

Pseudococcidae.

Two groups (in the wide sense) are involved. The species of the first are almost invariably associated with ants of the genera *Crematogaster* and *Pheidole*, and consist of the chief vector species, *Pseudococcus njalensis*, *Paraputo ritchiei* Laing,

Formicococcus tafoensis Strickland, and *Farinococcus loranthi* Strickland. They are characterised by relative sluggishness, very reduced legs, strong negative phototropism, and by an ovoviviparous habit. The first two species only are of importance. The second group consists of all other arboreal Pseudococcid species so far encountered and includes *P. citri* (Risso), *P. bukobensis* Laing, *P. concavocerarii* James, *P. longispinus* (Targ.), *P. celtis* Strickland, *P. hargreavesi* Laing, *Ferrisia virgata* (Ckll.), *Tylococcus westwoodi* Strickland, three species of *Pseudococcus* of doubtful position (groups of *masakensis* James, *gahani* Green, and *celtis*), and two species of *Phenacoccus* (one of which comes close to *hirsutus* Green). Members of this group are occasionally, but certainly not obligatorily, associated with ants; they have legs of normal pseudococcid length, and are generally far more active than members of the first group; they are mostly true egg-laying species, the eggs hatching several days after extrusion of the ovisac. Many are pests of introduced and ornamental plants on which they may be extremely common, nearby cacao being but little affected.

The identities of most of the above named species were established by the writer in 1947, and the most important fact that remained to be elucidated for the purpose of quantitative field work was the amount of variation that could be expected within the very variable species *Pseudococcus njalensis*. An attempt was accordingly made to see whether *P. njalensis* exhibited any consistent morphological variations such as might lead to the erection of valid sub-species.

Six groups, each consisting of 30 randomly selected mature adult female specimens, were cleared in 10 per cent. potassium hydroxide, stained in acid fuchsin (Morrison's formula), and mounted in balsam on microscope slides. The length of each specimen, as well as that of its antennae, was then measured by means of a micrometer scale, and counts were made of the total number of cerarian spines. The six groups were as follows:—

- (1) Thirty specimens from eight collections made from cacao in the dry areas of the Afram Plains and British Togoland.
- (2) Thirty specimens from 16 collections made from cacao in the main Eastern Province cacao area.
- (3) Thirty specimens from one colony on a cacao pod at Tafo.
- (4) Thirty more specimens from the same colony as (3).
- (5) Thirty specimens from nine collections from cacao made in the Tafo district of the Eastern Province.
- (6) Thirty specimens from ant domatia inside the hollow stems of the myrmecophyte *Canthium glabriflorum* Hiern.

The results of these series of measurements are given in Table I. It is clear from these that there is a very considerable range of variation within the *P. njalensis* group itself, but that the variation within collections from one district (i.e. Tafo), is less marked than between collections from widely separated areas, or from such restricted habitats as *Canthium* domatia.

It can hardly be said, however, that there is justification for erecting new sub-species, except possibly for the *Canthium* group.

Lecaniidae.

There is one Lecaniid species the identity of which is obscure (*Ceroplastes* sp. H.6037 in the WACRI Collection), but which, according to Dr. Hall, falls near to the group of *zonatus* Newstead. This species is sporadically common on cacao, and is the only one of the 12 Lecaniid species so far recorded from West African cacao that might become a local pest. All of the other species are either rare, or prefer food plants other than cacao, and are of no importance in the present work.

TABLE I.

Variation in *P. njalensis* colonies.

Group	Group means in order of magnitude	Standard error	Significant difference between mean.
(a) <i>Length of mounted specimens, millimetres.</i>			
5.	2.16	0.37	
4.	2.16	0.22	
3.	2.03	0.22	p.0.01 = 0.17
1.	1.89	0.21	p.0.05 = 0.13
2.	1.88	0.24	
6.	1.59	0.33	
(b) <i>Numbers of cerarian spines (all cerarii).</i>			
3.	162	8.50	
4.	160	7.10	
5.	145	5.92	p.0.01 = 13.7
2.	131	6.19	p.0.05 = 10.4
1.	127	5.47	
6.	99	5.46	
(c) <i>Antennal lengths, units.</i>			
1.	996.8	66.1	
4.	944.5	52.4	
3.	934.7	43.4	p.0.01 = 41.6
5.	922.7	71.0	p.0.05 = 31.6
2.	902.5	63.1	
6.	803.1	74.5	

Stictococcidae.

Central Africa is undoubtedly the original home of this family of scale insects. The monogeneric nature of the family, and the highly variable nature of three of the species (*Stictococcus multispinosus* Newst., *S. dimorphus* Newst., and *S. diversiseta* Silv.), renders it difficult to study. By far the most important cacao scale locally is *S. sjostedti* Ckll., and as this is probably the most stable species in the group, no difficulty has been encountered in making accurate field determinations of it. The characters separating *multispinosus*, *dimorphus* and *diversiseta* are unsatisfactory, and all three species are of very similar habit. Consequently they have been bulked together as *S. multispinosus* (sens. lat.) together with *S. gowdeyi* Newst., which is a characteristic and stable species, but not of sufficient importance to be treated separately.

Diaspididae.

Eight Diaspid species have been taken on cacao in the Tafo region in the course of the present work. They include *Aspidiotus destructor* Sign., *Aonidiella replicata* (Lind.), *Hemiberlesia palmarum* (Ckll.), *H. cyanophylli* (Sign.), two undetermined species of *Aspidiotus*, a *Pseudonidia* (group of *baikae* Newst.), and a *Selenaspidus* sp. Not one of these species is common enough to be of more than academic interest.

Margarodidae.

There are four Margarodid species associated with Gold Coast cacao. Three of them have been named. The fourth species may in fact turn out to be a complex of two closely related species of *Steatococcus*, close to *gowdeyi* Newst. These are referred to henceforth as *Steatococcus* spp.

Only the members of the Pseudococcid series have so far been proved to be vectors of swollen shoot, but the other species have been included in the present work primarily because the Coccoidea as a group are important plant pests, and little extra work was involved in obtaining quantitative data on them.

Formicoidea.

There are more than 70 species of ants associated with cacao and the cacao Coccids in the Gold Coast. The taxonomy of many of these is obscure, and a few notes are therefore appended to clarify the identities of those species which have proved to be of major importance.

Myrmicinae.

(1) *Crematogasterini* (consisting of the single genus *Crematogaster*).

Wheeler (1922) cites the subgenera *Decacrema*, *Oxygyne*, *Nematocrema*, *Orthocrema*, *Sphaerocrema*, *Atopogyne* and *Crematogaster* as being Ethiopian or occurring commonly in Central Africa. There is, however, considerable difference of opinion amongst taxonomic myrmecologists as to the correct interpretation of the systematic keys of Santschi (1918) and Wheeler (1922) to the *Crematogasterine* subgenera.

Representatives of the subgenera *Crematogaster*, *Sphaerocrema*, *Atopogyne*, *Nematocrema*, *Decacrema* and *Orthocrema*, as understood by Wheeler, have been taken in association with mealybugs in the present work. The individual species vary considerably in habits, and it has proved necessary, for purposes of analysis, to group certain species together in relation to their field behaviour. It is of some interest that this arbitrary grouping conforms reasonably well with some of the established subgeneric groups based on taxonomic principles, and it is hence possible to define the limits of the groups used in this paper with some accuracy. The following subgeneric groupings of the genus *Crematogaster* based on Wheeler (1922), are associated with Gold Coast cacao Coccids:

Sphaerocrema group. Workers only. Antennae 11-jointed, antennal club 2-jointed; epinotal spines of normal size; frontal carinae well developed; pronotum unarmed; petiole broadened in front, trapezoidal, sometimes truncated or rounded, entire; postpetiole always entire, without a median furrow; meso- and metanotum usually strongly punctate laterally; top of head, thorax, and entire abdomen usually shining; nests invariably arboreal, under flakes of bark, or in the rotten stump of a broken branch, or a *Loranthus* canker, with entrances through old wood-boring beetle holes; little carton used in nest manufacture*; almost invariably coccidophilic.

The species included in this group are *Crematogaster striatula* Emery, *C. luctans* Forel, *C. kneri* Mayr, *C. boxi*, Donis. and two undetermined species, one of which is very close to, and probably a variety of, *striatula*.

Crematogaster (sensu stricto) group. Workers only. Antennae 11-jointed, antennal club 3-jointed; epinotal spines of normal size; frontal carinae well developed; pronotum unarmed; petiole broadened in front, trapezoidal, truncated, or rounded; postpetiole always with a well defined median groove or furrow throughout its entire length; meso- and metanotum strongly punctate laterally; top of head, thorax, and abdomen matt rather than shining; nests invariably arboreal, usually under flakes of bark; little carton used in nest manufacture; almost invariably coccidophilic. This group includes only one, undetermined, species, *Crematogaster* sp. F.257 in the WACRI Collection.

* "It is perhaps advisable to clarify the use of the word 'carton.' Wheeler (1906) refers to carton tents of *Crematogaster lineolata* Say as '... structures which consist of agglutinated earth or vegetable detritus.' Subsequently, in his 'Ants of the American Museum Congo Expedition,' Wheeler refers (page 150), to the *Crematogasterine* habit '... of making carton nests is best seen in the tropical species, but traces of it survive even in the species inhabiting temperate regions, such as the North American *C. lineolata* Say.' Locally, the carton used in tent and nest manufacture appears to be identical in some species (e.g., in *C. boxi*), but in others, such as *C. striatula* and *C. africana*, it appears that two forms of carton are produced, the nest type being hard and brittle, the tent type being more often of felted vegetable debris markedly less brittle than that used in nest manufacture. For want of a better word, however, the writer has followed Wheeler in the present paper, and uses 'carton' qualified by nest or tent for both types of building material."

Atopogyne (*sensu lato*) group. Workers only. Antennae 11-jointed, antennal club of 3 or more joints; epinotal spines of normal size, usually blunt, and directed backwards and slightly downwards; petiole broadened in front; postpetiole merely impressed behind, not entire; basic sculpture densely striate; body generally dark in colour, deep brown to black, not shining, though in one species the abdomen is a shining yellow in colour. Nests invariably arboreal, of strong but brittle carton, on the trunks of trees; occasionally coccidophilic; most species can be found sporadically attending Lecaniids as distinct from Pseudococcids, but *C. africana* is an avid mealybug tender.

The species included in this group are *Crematogaster africana* Mayr, including its numerous varieties and sub-species, many of doubtful validity, *C. buchneri* Forel, *C. curvica* Donis., *C. depressa* (Latr.), *C. jullieni* Sants., *C. stadelmanni* Mayr, and four undetermined species.

All other subgenera. The easily identifiable subgenera *Decacrema* and *Orthocrema* (one species of each), and three species, the subgeneric placement of which is obscure, are included here. These ants are not sufficiently common to be of any importance in the present work.

(2) *Pheidolini.*

The tropicopolitan *Pheidole megacephala* (F.) is abundantly common in Gold Coast cacao farms and no attempt has been made to distinguish between its numerous varieties and sub-species. In addition, there is a group of ten *Pheidoline* species which have been taken sporadically, associated with mealybugs, but which are individually so rare that subgeneric or specific placement is of no importance.

(3) *Other Myrmicinae.*

Eighteen other ant species referable to the MYRMICINAE have been taken associated with the mealybug virus vectors. These include *Macromischoides aculeatus* (Mayr), *Atopomyrmex mocquersyi* E. André, *Paedalgus termitolestes* Wheeler, *Catantolus parallelus* F. Smith, two undetermined species of *Catantolus*, *Monomorium* (*Xeromyrmex*) *bicolor* Emery, *M. floricola* (Jerd.), *Meranoplus nanus* E. André, an undetermined species of ? *Xiphomyrmex*, *Tetramorium simillimum* (F. Smith), one undetermined *Tetramorium*, two undetermined *Solenopsis* spp., and a group of four species the generic placement of which is obscure.

(4) *Dolichoderinae.*

A group of five species, one of which is *Technomyrmex detorquens* (Wlk.), the other four all being members of, or very close to, *Tapinoma*. All are henceforth referred to as *Tapinoma* spp. These ants are of importance only so far as seedling cacao is concerned, and are all soil nesters.

(5) *Formicinae.*

Represented by the common *Oecophylla longinoda* (Latr.), with its variety *fusca* (Emery), four true Formicine species, (two of *Acantholepis*, and two of *Nylanderia*), and ten species belonging to the Camponotine. The Camponotine group collectively is of some importance, and has been split up as follows: *The Camponotus group.* *Camponotus* (*Myrmopiromis*) *chrysurus* Gerst., and two undetermined *Camponotus* spp. *The Polyrhachis group,* comprising *Polyrhachis* (*Myrma*) *laboriosa* F. Smith, *P. militaris* (F.), *P. revoili* E. André, *P. fissus* Mayr, three undetermined species of *Polyrhachis*, and *Phasmomyrmex polyrhachioides* (Emery). All of the latter group are arboreal.

(6) *Other Ants.*

A group of essentially predacious ants has been taken from time to time on cacao, probably as chance migrants onto seedlings or tree trunks from their normal habitat in the litter. The group includes *Tetraponera* (*Sima*) *anthracina* (Sants.), *Dorylus* (*Anomma*) *nigricans* Ill. (the common "driver ant"), *Aenictus asperivalvus* Sants., *Platythyrea conradti* Emery, *Odontomachus haematoda* (L.) and two undetermined species of *Anochetus*.

From the above list it will be seen that at least 75 ant species are associated with cacao in the Gold Coast. Of these the members of the *Crematogaster* (*Sphaerocrema*) and (*Crematogaster*) groups, the *africana* complex (of *Atopogyne*), and the bulked Pheidoline species are of considerable importance as mealybug tenders. Of the other species, indications are that *Oecophylla longinoda*, *Macromischoides aculeatus* and the *Polyrhachis* group are important in so far as they are negatively associated with mealybug tending ants, and hence with mealybugs.

Parasites and Predators of the Coccoidea.

The normal percentage of parasitism of the vector mealybugs in the field is extremely low (Posnette & Strickland, 1949), and some evidence is available to indicate that the associated ant species are at least in part responsible for this state of affairs.

Thirteen Hymenopterous parasites have to date been bred from *Pseudococcus njalensis* locally. They include *Anagyrus pullus* Comp. (the commonest species), *A. beneficans* Comp., two undetermined *Anagyrus* spp., *Leptomastix bifasciatus* Comp., *Tropidophryne melvillei* Comp., *Neodiscodes martinii* Comp., *Cheiloncurus carinatus* Comp. (possibly a hyper-parasite), two species of *Clausenia*, both new to science, which it is hoped will be described shortly, a species of the genus *Parateletracnemoides*, and two undetermined Encyrtids.

The only other parasites reared to date are an *Anagyrus* sp. and *Leptomastix bifasciatus* from *Pseudococcus citri*, *Leptomastidea abnormis* (Gir.) from *Ferrisia virgata*, and a series of *Pachyneuron* from *Stictococcus sjostedti*.

P. njalensis, being almost invariably ant attended, does not suffer to any extent from the ravages of predatory insects. The most important predator (and the same applies to *P. citri*), is a small red Cecidomyiid midge, the larvae of which suck the body fluids of the mealybugs, and pupate in cells made of the felted wax and debris from the bodies of their hosts. One larva may feed on several mealybugs in the course of its development. Two larval Coccinellids, *Platynaspis higginsii* Crotch, and an undetermined species of *Scymnus*, have been taken attacking young *P. njalensis* and *P. bukobensis* in the field. In addition, the common scavenger *Attagenus piceus* Oliv. has been taken as larvae inside ant tents. R. G. Donald has collected two species of mites which are evidently closely associated with *P. njalensis* colonies, one being an *Aleuroglyphus* sp., possibly a scavenger, the other an *Allothrombium* sp., a genus with truly ectoparasitic larvae. One predacious spider has been taken from *P. njalensis* colonies. It is a species of *Cheiracanthum*, and it has also been taken as predacious on nymphs of *Icerya nigroareolata* Newst. Attendant ants particularly of the *Atopogyne* group, are sporadically predacious on mealybug crawlers.

Predators of the other Coccid species are little better known. At least one species of the Noctuid genus *Eublemma* has been taken locally attacking *Stictococcus sjostedti*, this scale also being commonly attacked by a Lycaenid of the genus *Spalgis*. Two species of predacious spiders have also been taken in association with *S. sjostedti*; they are *Myrmarachne hesperia* Simon, and *M. near foesinex* Simon, the latter species having been taken inside a nest of *Oecophylla longinoda* which were attending the scales on a nearby twig.

BIOLOGICAL NOTES.

Coccoidea.

In order to indicate the relative importance of the various Coccid species involved in the present work, Table II has been drawn up. This table has been compiled from figures obtained from nearly 3,000 randomly selected cacao trees, and 25,000 cacao pods, and is thus referable only to cacao as a host plant.

TABLE II.

Relative abundance of Coccid species on cacao at Tafo.

Coccid species	Total bugs from random collections
<i>Pseudococcus njalensis</i>	273,750
<i>P. citri</i>	2,737
<i>P. buhobensis</i>	82
<i>P. concavocerarii</i>	67
<i>P. longispinus</i>	18
<i>P. cellis</i>	1
<i>Ferrisia virgata</i>	71
<i>Paraputo ritchei</i>	9
All other Pseudococcid species	9
<i>Stictococcus sjostedti</i>	199,857
All other Stictococcid species	5,451
<i>Ceroplastes</i> spp.	2,843
All other Lecaniid species	95
<i>Steatococcus</i> spp.	1,240
All other Margarodid species	229

The figures for the non-Pseudococcid forms noted are referable to data from the mature trees only, and are exclusive of pod counts. As such they are only strictly comparable to a total of 189,267 *P. njalensis*, 1,939 *P. citri*, and 219 other Pseudococcid species. Nevertheless, it is clear that *P. njalensis* and *S. sjostedti* form the two dominant cacao Coccid species, and together account for over 97 per cent. of the total.

It is again clear that *P. njalensis* is by far the most important cacao Pseudococcid present in the Gold Coast, and that *P. citri* comes a very poor second in order of dominance.

A third fact of some interest is that, taking the mature tree figures only, all Pseudococcid species total 191,424 bugs, and all Stictococcid species total 205,308 bugs, indicating virtually the same degree of environmental adaptation for both groups.

The mature tree data are referable to 2,880 randomly selected cacao trees, from which 401,140 Coccids of the groups noted in Table II were collected. This figure gives a mean Coccid infestation rate for Gold Coast cacao (exclusive of Diaspid scales), of 140 individuals per tree, or, for Pseudococcid forms only, of 66 per tree. It is abundantly clear that the Coccids cannot be described as important cacao pests in their own right.

(1) *Pseudococcus njalensis*.

Life-history. This species is ovoviviparous, adult females producing eggs which hatch within a few minutes of extrusion. Extrusion of the egg takes 15-20 minutes, and at the same time very thin, glassy, wax filaments (a rudimentary ovisac), are produced from glands surrounding the genital opening. When the egg has been completely extruded, the "shell" (a thin yellow light-reflecting membrane surrounding

the young nymph), dries or evaporates, leaving the young "crawler" lying free. The complete drying process takes about 10 minutes. It is at this stage that the crawler begins to show signs of life. First, its fore legs are unfolded and stretched, the other legs following in order; all pairs of legs are flexed several times, the body simultaneously arching itself and stretching, and the antennae being unfolded and flexed. The newly born crawler then, very slowly and hesitantly, walks a few millimetres, and settles down for its first feed. About 45 minutes elapses between the first appearance of the egg to the time that the crawler walks. One adult female was observed to give birth to 3 crawlers over a period of $3\frac{1}{2}$ hours, 2 of which died within an hour of birth. In a number of cases (for example, under insecticidal stimulation), the egg capsules have been observed intact up to 2 hours after extrusion but in these cases the crawlers have probably died before extrusion. A mature female of *P. njalensis* normally produces 30 to 40 viable eggs over a period of up to 20 days.

In an exhaustive study of the sex ratios of five Pseudococcid species, James (1937) points out that "the male of *Pseudococcus* is co-equal in importance with the female for the propagation of the species . . ." and that "The relative abundance of the male sex varies greatly from species to species." He found that, in the case of the species with which he was working, the fecundity of the females gave a mean of roughly 180 (range: 110 to 239). *P. njalensis* differs from all of James' species in showing a strong parthenogenetic habit, and a much lower fecundity. James appears to use the term "fecundity" to mean "total progeny," and since he divides his "total progeny" into males and females, it is evident that he includes only those mealybugs on the experimental plants during the third instar, when males pupate and can be distinguished from female larvae for the first time. In the present paper, the term "fecundity" is used to indicate the maximum number of nymphs of either sex surviving after the first moult. Fifteen individual breeding tests were carried out in which no attempt was made to assess the proportion of the sexes. The results indicated that *P. njalensis* has a fecundity varying from 6 to 90, with a mean of 36.

Seventy-four cacao seedlings in individual bamboo pots in individual gauze cages were each infested with one mature adult female taken from a field colony of *P. njalensis* in order to determine the duration of the life-cycle. As soon as a female commenced to oviposit she, and all but one of the new born crawlers, was removed from the plant, the single remaining crawler being observed daily and notes made on its behaviour. The tests were carried out at room temperature in the laboratory, and no data on temperature or humidity are available. Table III gives the results of these tests.

TABLE III.

P. njalensis, life-cycle.

Stage	Mean No. days	Range, days	Number of trials
1st instar	7	4-13	74
2nd instar	5	3-10	31
3rd instar	7	5-9	21
Adult to oviposition ..	23	18-30	8
TOTALS ..	42	30-60	8

It will be noticed that in the 74 individual breeding tests, only on eight occasions did a new born crawler remain on its original host plant throughout its life-cycle. There is marked activity on the part of the nymphs, particularly on completion of ecdysis, a fact which is evident from the figures given above, and which is of some importance when considering the dissemination of swollen shoot virus.

Females live for 10 to 14 days after oviposition has been completed, before dying or becoming moribund but on one occasion, a female survived for 65 days without laying any eggs. This is of interest since, in Table III, the eight females that survived to produce eggs 18 to 30 days after they emerged from the third instar could not have been fertilised by males, and must have reproduced parthenogenetically. The 65 day adult may, therefore, have been unable to produce in this manner, an indication that the parthenogenetic habit of *P. njalensis* may be in some way restricted.

Host Preferences. *P. njalensis* has been taken from 110 species of indigenous and introduced plant species in the Gold Coast (Hall, 1945; Strickland, 1947; Anon., 1948). Most of these host records are from single collections from the plant host concerned, so little quantitative information can be obtained on generic or specific preferences. It has, however, been possible to group into their respective plant families the commoner plants from which collections of Coccid material have been made, and thus obtain some quantitative information on host preferences.

Table IV summarises this information. It should, however, be pointed out that the Bombacaceae consists of 83 examinations of *Ceiba pentandra*, the Silk Cotton tree, and 4 of *Bombax buonopozense*. The Rubiaceae are biased because 95 per cent. of the *Canthium* trees examined (and all of the mature specimens) were infested with *Crematogaster africana* in association with *P. njalensis*. The Sterculiaceae excludes *Theobroma*.

TABLE IV.
Food preferences of *P. njalensis*.

Plant Family	Times examined	Times with <i>P. njalensis</i>	
		Number	% infestation
Bombacaceae ..	87	8	9.2
Euphorbiaceae ..	42	7	16.7
Leguminosae ..	49	10	20.4
Moraceae ..	46	6	13.0
Rubiaceae ..	51	31	60.8
Sterculiaceae ..	126	12	9.5
Solanaceae ..	23	3	13.0
Urticaceae ..	41	1	2.4
TOTALS	465	78	16.8
<i>Theobroma cacao</i> ..	2,880	1,180	40.9

Approximately 17 per cent. of the wild plants growing in Eastern Province cacao farms are infested with *P. njalensis*, as distinct from 41 per cent. of the cacao. Although this mealybug clearly prefers cacao, it was considered that the myrmecophyte *Canthium* should be investigated in greater detail in view of the invariable association of *P. njalensis* with the ants inhabiting this plant. In one *Canthium* felled and dissected in its entirety, 7,498 mealybugs were found. Of these 6,864 were in canopy domatia, 178 were in secondary branch domatia, 456 were in main trunk domatia, and 317 were on the outer surface of the tree (leaves, 177, trunk and branches 140). Ten further *Canthium* trees were subsequently felled, and, on the basis of the numbers of mealybugs in random 1-foot long branch sections, the mean population was computed as 10,697 mealybugs per tree, with a range of 2,356 to 18,683. Since, however, *Canthium* is rarely found at a density exceeding 1 per 2 to 3 acres (as distinct from 500-600 cacao trees per acre), this tree is of relatively minor importance as an alternative food plant.

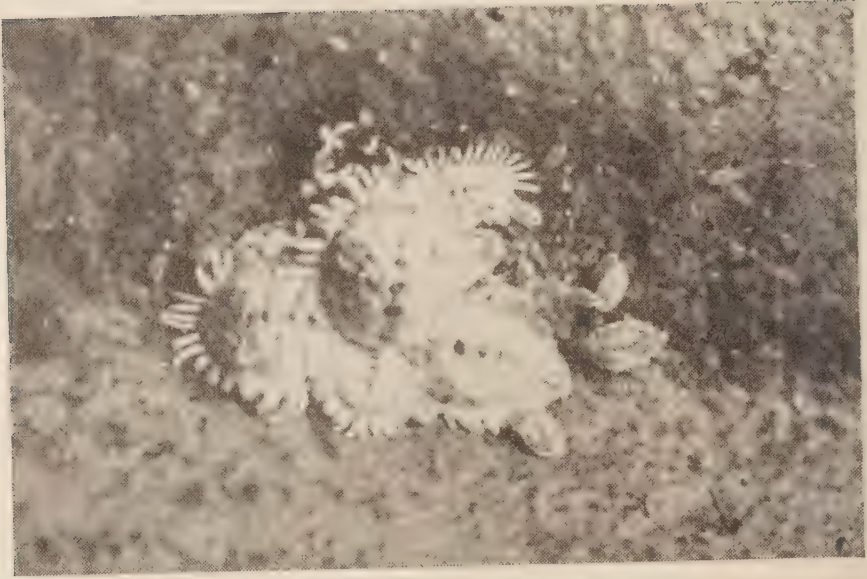


Fig. 1 Adult females and nymphs of *P. njalensis* in compact colony on a cacao pod.

Migration and Dispersal. It has already been pointed out that there is a considerable amount of migration from established colonies on the part of freshly moulted nymphs. In addition, young adults are easily disturbed by external stimuli, and will walk quite long distances in their search for a more peaceful feeding site. The maximum speed at which a young adult *P. njalensis* appears to be able to walk has been determined from a series of laboratory tests at approximately 5 feet in 20 minutes, but more normally such migration is taken in stages, and it is unusual to find a young adult walking more than a few inches along a branch, or cacao pod, before it settles down for a rest or feed. This behaviour is undoubtedly largely attributable to its relatively small legs.

It was clearly important to determine how far litter migration of the type noticed for *P. bukobensis* (Strickland, 1947), assisted in the dispersal of *P. njalensis*, and 3 experiments were set up with this end in view.

First, it was necessary to determine how long mealybugs remained on a tree once it had been felled and whether it was possible for vectors on a felled virus infected tree to migrate over the litter and establish themselves on nearby healthy cacao trees. Some individuals could still be found 18 days after felling, on heavily infested mature trees (with more than 500 mealybugs, and girth 18–20 inches at 1 foot above ground), but they were entirely absent by the 24th day. All the specimens on less heavily infested, and smaller, trees (with 50–100 mealybugs, and girth 10–12 inches), had disappeared by the 11th day after felling. This was to be expected since the larger the tree, the longer it would take to desiccate. As several of the trees felled in this experiment were virus infected, an opportunity was available to ascertain how long the virus remained available to the mealybugs after felling.

It was known at the time that infective vectors lost their infectivity if starved for more than 36 hours, and the experimental results indicated that vectors could remain infective for not more than 14 days after the host tree had been felled. It was thus possible to ignore, from the economic standpoint, those mealybugs that remained established for longer than this period.

When an infested cacao tree is felled, some of the mealybugs get thrown off the tree as it falls. Others are immediately carried off by attendant ants, but many remain *in situ*, or at the most wander about on the felled trunk and branches before settling down again.

The second experiment was designed to ascertain to what extent unassisted and ant-assisted migration and establishment takes place after the mealybugs have left their fallen host.

A release post was set up in the middle of a plot of mature cacao, 4 feet from the nearest cacao tree. Fifty-six cacao trees within a radius of 60 feet of the release post were carefully examined from a height of 3 feet down to ground level, and any mealybugs in cracks in the bark were removed and killed. The trees were then thickly ringed with banding grease at the 3-foot level. Batches of about 500 active young adult mealybugs were obtained from naturally infested cacao in the field, and were set down on the litter at the release post. Observations were made at frequent intervals on the trunks of the banded trees and on the mealybugs remaining at the release post, and the results are summarised in Table V.

TABLE V.

A. Unassisted Migration.

Test No.	Mealybugs put down	After 3 hours	Subsequent behaviour
1.	400	Attack by <i>Pheidole megacephala</i> from soil nest	1 adult mealybug only in litter after 7 days. No infestation of the cacao.
2.	600	No <i>Pheidole</i> . Fine weather.	Heavy rain 3rd day. 28 mealybugs still in litter 4th day. No establishment, all bugs disappeared by 7th day.
3.	650	<i>Pheidole</i> attack. Fine weather.	No mealybugs in litter on 4th day. No establishment.
4.	500	No <i>Pheidole</i> attack. Heavy rain.	106 mealybugs in litter on 4th day. No establishment, and all bugs disappeared by 10th day.
5.	500	No <i>Pheidole</i> attack. Heavy rain.	1 adult on litter after 24 hours. After 11 days, 1 adult established on bole of cacao tree 8 feet from release post.
6.	500	No <i>Pheidole</i> attack. Fine weather.	10 adults on litter after 24 hours. None present after 5 days. No establishment.

In the six trials in which mealybugs were put down without attendant ants, only one of the 3,150 young adults used established itself on the bole of a cacao tree 8 feet from the release post. In the case of the attended mealybugs (Table VI), pieces of carton nest of *Crematogaster africana* were placed at the release post, and the mealybugs

were put down evenly around the pieces of nest. One only of 2,010 such young adults succeeded in establishing itself on the bole of a cacao tree 4 feet from the release post. In both cases, Pheidoline ants already nesting in the soil attacked and carried off many of the mealybugs, and in the latter case the Crematogasterine ants were themselves attacked both by species of *Pheidole*, and of *Crematogaster* and *Oecophylla* when they tried to establish themselves on the nearby cacao trees. It is thus clear that there is little direct migration and successful establishment of mealybugs over the litter and on to fresh hosts.

TABLE VI.

B. Ant-assisted Migration.

Crematogaster africana used throughout as attendant ant.

Test No.	Mealybugs put down	After 1 day	Subsequent behaviour
1.	500	1 bug established on seedling 2 ft. 6 in. from release post. 3 adults still on litter.	<i>C. africana</i> attempted to colonise two cacao trees, but driven off by <i>C. depressa</i> , which already had nests on these trees. By 8th day, <i>C. africana</i> nest swarming with <i>Ph. megacephala</i> , and deserted by <i>C. africana</i> .
2.	400	6 bugs still on litter. <i>C. africana</i> ants being attacked by <i>Oecophylla</i> , which had come down from canopies of 2 nearby trees.	Ants still fighting on 5th day. On 6th day, 1 adult mealybug established on tree bole 4 feet from release post. On 8th day, <i>C. africana</i> ants vanquished and <i>Oecophyllas</i> swarming over nest carton. No mealybugs on litter.
3.	500	30 mealybugs on litter. <i>Pheidole</i> ants swarming over them. <i>C. africana</i> ants being attacked by <i>C.257</i> ants that had come down from adjacent cacao trees.	After 2 days, only 1 mealybug still on litter. After 3 days, <i>C. africana</i> ants completely vanquished by <i>C.257</i> and <i>Pheidole</i> ants, and nest carton deserted.
4.	500	44 mealybugs and few <i>C. africana</i> ants present.	1 mealybug on litter on 2nd day. <i>C. africana</i> ants all disappeared. No bugs on 3rd day.
5.	Pod with 60 adults.	55 adults on pod, attended by <i>Pheidole</i> ants from nearby soil nest.	On 4th day, 46 adults still on pod, but 11 had moved to the underside, and were being covered by an earthen tent that the <i>Pheidole</i> ants were building. The pod had been cemented to a fallen leaf by the ants. No establishment on cacao.

The ant-assisted migration tests described above are, however, open to the criticism that the ant species already dominant in the release post area invariably attacked the introduced ant attendants. It was considered that this criticism could be overcome by using as release posts naturally infested cacao and *Canthium* trees felled in the field in an area in which the attendant Crematogasterine ant species were known to be dominant. The results of this experiment are summarised in Table VII.

TABLE VII.

Migration from felled trees in the field.

Test No.	Tree felled	Surrounding cacao (number of trees)	Examination after felling, (days)	Cacao trees with	
				Ants	Mealybugs
1.	<i>Canthium.</i>	12	6	1	1
	"		15	3	-
2.	"	31	2	18	-
	"	"	20	9	2
	"	"	27	10	2
3.	Cacao.	20	3	-	-
	"	"	6	2	-
	"	"	21	-	-
4.	"	18	3	6	-
	"	"	18	6	1
	"	"	25	7	1
5.	"	15	3	-	-
	"	"	18	1	1

It will be noted that 8 of the 96 surrounding cacao trees became infested with mealybugs from the felled trees during the experimental period. One tree (4 feet from felled tree) became infested after 6 days, two (4 feet and 10 feet away) 18 days after felling, two (12 feet and 18 feet away) 20 days after felling, one (8 feet away) after 25 days, and two (both 10 feet from the felled tree) at about 27 days.

It is thus clear that ant-assisted migration and successful establishment does take place from felled mealybug-infested trees, but that such establishment is on a small scale, and mostly does not take place until after any virus in the felled tree has died. It is also apparent that such migration takes place only over relatively short distances, and that *Crematogaster africana* appears to be far more concerned in re-establishing itself on a new host than in re-establishing its mealybug associate.

The writer has already shown (Strickland, 1950), that there is an appreciable amount of wind or air current dispersal of young nymphs in the dry season, when air movements are largely conditioned by the Harmattan. In addition to this direct form of dispersal, it seems very likely that infested leaves, carried by air currents, also play a part in the dispersal of *P. njalensis*.

As far as is known, the attendant ant species do not transport young mealybug nymphs during the marriage flight, as in other parts of the world (Weber, 1944; Bünzli, 1935).

There is little doubt that the interlocking canopy of West African cacao is ideal for the dispersal of both *P. njalensis* and swollen shoot. A mealybug has only to walk perhaps half-an-inch to find itself on a leaf belonging to a new host tree. Virus can move through the tree, so that another mealybug many feet away from the initial infestation point can likewise inoculate a third tree with the virus by walking a very short distance indeed. *P. njalensis*, closely associated as it is with certain arboreal ants, need never, and in fact very rarely does, come anywhere near the ground. Yet, it can spread itself through mile after mile of standing cacao by the simple expedient of canopy migration without undergoing the perils of litter migration.

Distribution. Table VIII shows the distribution of the 189,267 *P. njalensis* noted on page 731 on their cacao hosts. The table is compounded from the bulked results from 1,180 infested trees examined.

TABLE VIII.

Distribution of *P. njalensis* on cacao at Tafo.

Feeding site			Number of mealybugs	As per cent. of total
Canopy	{ Leaves	..	1,434	0.8
	{ Shoots	..	142,910	75.5
	{ Bark	..	17,415	9.2
	{ Pods	..	3,274	1.7
Trunk	{ Bark	3,128	1.7
	{ Pods	15,008	7.9
Chupons	6,098	3.2
TOTALS	189,267	100.0

Over 87 per cent. of the total mealybugs were taken in the canopy, 15 to 20 feet above ground level (*cf.* the *Canthium* figures on page 733). The new canopy shoots are the sites preferred most, with cracks in the bark, pods, and pod stalks, being the next most favoured.

P. njalensis is essentially a West African species. It was originally described from Njala in the forest belt of Sierra Leone, and has been taken subsequently from localities in Liberia, Ivory Coast, Gold Coast, Togoland, Western Nigeria, and the British Cameroons. It is a forest species, and becomes more scarce as one proceeds northwards. The most northerly record from the Gold Coast is from Wenchi, in Ashanti, on the edge of the Guinea Savannah country. The species has not as yet been recorded further east than Tombel in the British Cameroons (2 out of 12 Pseudococcid collections received = 16.7 per cent.). Table IX gives the relative abundance of *P. njalensis* in various parts of West Africa, as calculated from the total number of collections of Pseudococcids received from all host plants from the various areas.

TABLE IX.

Distribution of *P. njalensis* in West Africa.

Locality	Pseudococcid collections received	<i>P. njalensis</i> collections received	
		Number	As % of total
Sierra Leone, Ivory Coast and Liberia ..	29	20	69.0
Gold Coast, west of Akwapim Ridge ..	721	341	47.3
Gold Coast, east of Akwapim Ridge and Togoland	74	36	48.6
Western Province of Nigeria	107	21	19.6

It is clear that *P. njalensis* becomes less common as one proceeds eastwards of the type locality. How far the dry area of Togoland acts as a barrier to the eastward spread of the species is unknown. It is known, however, that there is very little cacao in this dry region, and further that the cacao in western Nigeria differs markedly from Gold Coast cacao in that it is grown without forest shade. As will be shown below *P. citri* appears to be the dominant cacao mealybug in Nigeria.

(2). *All other Pseudococcid species.*

Life-histories. Studies on the Pseudococcid species other than *P. njalensis* have had to be restricted to a minimum. Cotterell (unpublished data) gives the nymphal life of *P. citri* on cacao as 32 to 38 days. The writer watched the development of a colony of this species on a pod in the field, and noted that second generation females were depositing their ovisacs 5 to 6 weeks after they themselves had hatched out. The egg stage lasts 3 to 4 days in the Gold Coast. Cotterell found the egg stage of *Ferrisia virgata* lasted 1 day, the nymphal life 21 to 35 days, though two cases were noted in which the nymphal life apparently lasted 87 and 99 days respectively. The period from adult to egg laying lasted 20 to 30 days. Cotterell's figures gave periods "from egg to egg laying" of 41, 44, and 47 days, with a mean of 44, much the same as *P. citri* and *P. njalensis*. *P. bukobensis* is another egg laying species that produces a complete ovisac, the egg hatching in 3 to 4 days, and the nymph having a life of about 3 weeks. Mealybugs of the *Formicococcus-Paraputo* group are ovoviviparous, but no information as to their life-histories is available.

Host Preferences. The species, other than *P. njalensis*, do not seem to prefer cacao as a food plant. *Paraputo*, for example, is often associated with *Cola* spp. but is rarely found on cacao. *Formicococcus* seems to prefer *Ceiba* to cacao. *P. bukobensis* oviposits and feeds readily on *Trema guineensis* (Ulmaceae), and will feed on cacao, but only very rarely oviposits on this host. *P. longispinus* reaches peaks of infestation on many ornamental plants (e.g. *Aristolochia*) that are never equalled on adjacent cacao. *F. virgata*, similarly, has been found infesting *Leucaena* at major pest proportions, whereas the cacao growing amongst the *Leucaena* was almost completely neglected. *P. citri*, exhibiting no very strong host preferences, is second only to *P. njalensis* in importance as a cacao mealybug. In fact, the column referable to *P. citri* in Table X below is remarkable for its degree of agreement with Table IV, indicating that this species and *P. njalensis* have rather similar food plant tastes, although *P. njalensis* is certainly more commonly found on Rubiaceae plants and on cacao than *P. citri*.

TABLE X.

Food plant preferences of "other Pseudococcid species."

Plant Family	<i>P. citri</i>		<i>Ferrisia virgata</i>		<i>Paraputo ritchiei</i>		All other spp.		With any Pseudococcid spp. (including <i>P. njalensis</i>)	
	Times	%	Times	%	Times	%	Times	%	Times	%
Bombacaceae ..	5	5.8	1	1.2	—	—	18	20.8	32	37.1
Euphorbiaceae ..	9	21.4	6	14.2	—	—	4	9.7	26	61.9
Leguminosae ..	8	16.3	10	20.4	1	2.0	5	10.3	34	69.4
Moraceae ..	11	23.9	3	6.4	1	2.2	6	12.6	27	58.7
Rubiaceae ..	5	9.8	—	—	—	—	6	11.7	42	82.3
Sterculiaceae ..	14	11.9	4	3.5	20	15.8	18	15.4	68	53.9
Solanaceae ..	4	17.4	1	4.1	—	—	5	21.6	13	56.1
Urticaceae ..	1	2.4	3	7.2	—	—	10	24.6	15	36.6
TOTALS ..	57	12.5	28	6.1	22	4.2	72	14.7	257	55.2
<i>Theobroma cacao</i> ..	312	10.8	17	0.6	—	—	73	2.5	1,335	46.4

The category "all other species" in Table X needs a little explanation. In the Bombacaceae, as has been pointed out on page 733, *Ceiba* is the dominant species, and the mealybugs in this category are almost exclusively *Formicococcus*. For the other plant families, *P. bukobensis*, *P. longispinus*, several undetermined species of *Pseudococcus*, and several species of *Phenacoccus* occur more or less equally, with the exception of the Sterculiaceae, where *Formicococcus* again becomes dominant. It is of considerable interest that *Paraputo* is virtually restricted to the Sterculiaceae,

and to *Cola* spp. (*chlamydantha* and *cordifolia*), within that family, particularly since both of these plant species have been found to be alternative hosts of swollen shoot, and that *Paraputo* has been shown to be naturally infective when feeding on diseased *Cola* spp. in natural forest in the Western Province of the Gold Coast. It would seem possible that this mealybug is one of the vectors of virus from forest hosts to cacao, the virus then being spread amongst the cacao by the various species of *Pseudococcus*.

Another point of some interest in Table X is that the mean infestation rate for all Pseudococcid species on the wild food plants investigated appears to be of the order of 55 per cent., which is very similar to the mean cacao infestation rate.

It is probably safe to say that approximately half of the trees in the Gold Coast cacao belt are infested to a greater or lesser degree with one or other of the Pseudococcid species known or suspected as vectors of swollen shoot.

Migration and dispersal. The forms of dispersal noted for *P. njalensis* apply equally to the other species. There are, however, exceptions. *P. bukobensis*, for example, has legs twice as long as those of *P. njalensis*, and is more active at all stages in its life-history. *P. citri*, *longispinus*, *conconvocerarii*, and *F. virgata* likewise have longer legs, and are generally more active than *P. njalensis*. These species are not attended by ants to the same extent as *P. njalensis*, and consequently they may have to rely on their own powers of locomotion for dispersal. Quantitative estimates have shown that, on cacao, ant attendance ratios are approximately one unattended *P. njalensis* to 225 attended specimens, one unattended *P. citri* to five attended specimens, and one "other species" to 2.3 attended specimens. The large mealybugs of the *Formicococcus-Paraputo* group have legs of the same relative size as, or shorter than, *P. njalensis*. These species are usually ant attended, and are undoubtedly carried about by ants in the same way as the latter species. Wind dispersal is probably more common in such species as *P. citri* (which has a well developed ovisac that is easily wafted about by air currents), and especially *F. virgata* (the young crawlers of which rapidly develop two fine anal wax filaments which act as "sails") than in *P. njalensis*.

Distribution. *P. citri*, *P. longispinus*, and *F. virgata* are all common species of world wide distribution. The other species are essentially Ethiopian, *P. bukobensis*, *conconvocerarii*, *hargreavesi*, and *Paraputo* having been described from Kenya, Uganda, and Tanganyika. *Tylococcus* is another typically Ethiopian genus, the true species in the genus having been described from Madagascar and the Gold Coast. Species of *Formicococcus* and *Farinococcus* have been collected in the Neotropical region, and in Ceylon. Table XI gives an indication of the relative distribution of some of these species in West Africa.

TABLE XI.
Distribution of some Pseudococcid species in West Africa.

Locality	Collections of all Pseudococcid species received. Number	<i>P. citri</i>		<i>F. virgata</i>		All other species excepting <i>P. njalensis</i>	
		Number	%	Number	%	Number	%
Sierra Leone, Ivory Coast and Liberia	29	4	13.8	2	6.9	3	10.3
Gold Coast, west of the Akwapim Ridge	721	138	19.1	53	7.3	189	26.3
Gold Coast, east of the Akwapim Ridge, and Togoland ..	74	16	21.6	7	9.4	15	20.4
Western Province of Nigeria ..	107	55	51.4	14	13.1	17	15.9

Taken in conjunction with Table IX, Table XI is of some interest. First, whereas *P. njalensis* decreases in abundance from west to east, *P. citri* clearly increases in abundance as one moves eastwards, and, in Nigeria, becomes the dominant cacao mealybug. The same applies to *P. virgata*, suggesting that both of these forms prefer a drier climate to that prevailing in that wet cacao forest belt of the Gold Coast. So far as "all other species" are concerned, the Sierra Leone-Liberia-Ivory Coast figure of 10.3 per cent. is probably biased as no collections were received from these areas from forest trees, and thus there was little chance of collecting such forms as *Paraputo*. All of the mealybugs in this category are, in fact, species of *Pseudococcus*. The eastern Gold Coast and Togoland category of "other species" consists mostly of *P. concavocerarii*, a species that is relatively common in this area. There have been very few forest tree collections made in Nigeria, and so this category, also, consists largely of true *Pseudococcus* species.

In short, Tables IX and XI indicate that the virus vector complex is markedly different in Nigeria from that in the Gold Coast and westwards. In view of the possibility of biological control of the vector species, this is a fact of importance.

(3) All other Coccid species.

No biological work has been carried out on the non-pseudococcid cacao Coccids in view of their relative unimportance, and the fact that evidence to hand indicated that it was unlikely that any of the species named on page 727 would be shown to be vectors. Recent detailed tests have, for example, shown that *Stictococcus sjostedti* is not a vector of strain A virus. A considerable amount of quantitative field work has, however, been carried out, and this will appear more appropriately in a later paper.

Formicoidea.

ABUNDANCE.

It is not easy to assess accurately the abundance of social insects. A list has been given of the ant species (page 728) involved in the present work and, in Table XII, an attempt has been made to assess their abundance on the basis of random collections. In each case, a "collection" may be referable to a single specimen taken, perhaps, palpating a mealybug, or to a nest of several hundreds of individuals from a single tree. Collections have been made from cacao in all growth stages, and the classification is to a certain extent arbitrary. For example, *Pheidole* spp. sometimes nest in trees, but the bulk of the members of this genus were taken from seedling cacao, and were soil nesting. Hence, they have been included under terrestrial forms. Again, some of the Camponotine species nest in rotting logs or in the soil, but many are arboreal, and so the group has been included in both the arboreal and terrestrial categories.

TABLE XII.

Relative abundance of ant genera on cacao at Tafo.

1. Essentially arboreal genera.					No. of collections
(a) Coccid tenders.					
Myrmicinae	<i>Crematogaster</i>	1,990
	<i>Catantopus</i>	60
	<i>Atopomyrmex</i>	36
Formicinae	<i>Oecophylla</i>	1,029
	<i>Camponotus</i>	50
	<i>Polyrhachis</i>	262
(b) Non Coccid tenders.					
Myrmicinae	<i>Macromischoides</i>	317
	Other Myrmicine spp.	17
Ponerinae	<i>Anochetus</i>	3
	<i>Odontomachus</i>	1
Pseudomyrminae	<i>Tetraponera</i>	1
TOTAL arboreal collections					3,766

TABLE XII.—continued

2. Terrestrial genera.						No. of collections
(a) Coccid tenders.						
Myrmicinae	<i>Pheidole</i>	400
	<i>Tetramorium</i>	26
	<i>Paedalgus</i>	9
Dolichoderinae	<i>Tapinoma</i>	94
Formicinae	<i>Camponotus</i>	148
(b) Non Coccid tenders.						
Myrmicinae	<i>Solenopsis</i>	21
Formicinae	<i>Acantholepis</i>	148
	<i>Nylanderia</i>	4
Ponerinae	<i>Platythyrea</i>	27
Dorylinae	<i>Dorylus</i>	1
TOTAL terrestrial collections						878
Total Coccid tenders						4,104
Total non Coccid tenders						540
Total mealybug tenders						2,615
GRAND TOTAL: 4,644 collections.						

The distinction drawn at the foot of this table between coccid tenders and mealybug tenders is necessary because, for example, *Oecophylla*, and the other Formicine ants are commonly associated with Stictococcids, and rarely, if ever, with true mealybugs of the Pseudococcid series. As might be expected, the ant fauna of cacao is largely arboreal. Those terrestrial forms that are found on cacao, such as *Paedalgus*, occur either only on the trunks of mature trees or as migrant forms on young cacao up to 5 or 6 inches in girth where, for example, *Pheidole megacephala* will tend mealybug colonies whilst retaining its nest in the adjacent soil. It is also evident that over 88 per cent. of the ants on cacao are coccid tenders, the balance being essentially predacious, and that the true mealybug tending forms comprise just over 56 per cent. of the total cacao ant fauna.

The MYRMICINAE and the FORMICINAE are the two dominant tribes, with 2,876 collections and 1,641 collections respectively out of the total of 4,644 examined. *Oecophylla* is the dominant species, but *Crematogaster* is the dominant genus. Within the *Crematogasterini*, *C. striatula* is the dominant species, with 778 collections out of 1,776 collections that were determined specifically as distinct from generically.

HABITS.

(a) *Crematogasterini*.

(1). *Sphaerocrema* group. The dominant species in this group is *C. striatula*. It is invariably arboreal, building nests of vegetable carton often under a flake of bark, or at the end of a broken and dying branch. The carton is used to divide what was originally a free space into galleries and connecting passages. Usually, but not invariably, a queen and brood is present. The nests are always small in size (about $3 \times 2 \times 1$ ins.), and hard to see from the ground. Winged forms were present in a nest on one occasion in February, on two in April, and on six in May. It is thus clear that the species normally swarms in April or May (actual dates: 16th and 26th April, and 16th and 19th May), which is to be expected in the forest belt of the Gold Coast, where the rains have just broken at this time of the year. A variety of *C. striatula* is cited by Wheeler (1922) as inhabiting the inflated stipules of *Uragoga* sp., attending Coccids, at Leopoldville, Belgian Congo. The writer has taken *C. striatula* attending mealybugs under carton tents on the stems of *Octolobus spectabilis*, attending *Farinococcus loranthi*, with a nest in a *Loranthus* canker; attending *P. njalensis* and feeding on the leaf nectaries of *Cola chlamydantha* in primitive forest at Wiawso, in the Western Province of the Gold Coast; and with nests on *Blighia*

sapida, *Triplochiton scleroxylon*, *Newbouldia laevis*, *Terminalia superba*, *Carapa procera*, *Ficus exasperata*, *Ficus* sp., *Celtis* sp., *Ceiba pentandra*, *Mangifera indica*, *Discoglyprena caloneura*, and *Piptadenia africana*. The species is clearly well distributed on forest host plants of the mealybug vectors. It has been collected in Sierra Leone, Liberia, Ivory Coast, Gold Coast, Togoland, Dahomey, Western Nigeria and the Cameroons.

The next dominant species in the *Sphaerocrema* group is probably a variety of *C. striatula*, but pending expert opinions it is referred to as *Crematogaster* species F.361. It is less common than true *striatula*, but is almost identical in habit. The only forest hosts from which it has been taken are *Euntumia elastica*, and *Terminalia superba*, but it probably occurs on other species of the indigenous flora. Swarming takes place at the end of April. The only other *Sphaerocrema* worthy of individual mention is *C. luctans*, which is less common than either of the above-named species (88 collections to 778 of *striatula*). This species has been taken in association with *striatula*, attending the same colony of mealybugs on *Octolobus spectabilis*, and on *Cola* sp. It appears to be more common than *striatula* in Nigeria. The nuptial flight takes place at the end of April or early in May. The other species of the *Sphaerocrema* group are all rare, at least on cacao. One of them (*C. kneri*) was taken once attending Aphids, on an undetermined shrub in Togoland, which is of interest because the other species of the *Sphaerocrema* group have never been taken by the writer attending these insects.

(2). *Atopogyne* group. The dominant members of this group on cacao generally are *C. depressa*, and *C. buchneri*. On *Canthium*, however, and in cacao farms in which *Canthium* is common (more than two to the acre), *C. africana* becomes dominant. Ratios from random collections in relation to 778 *C. striatula* were 27 collections of *C. africana* and 218 of the *depressa-buchneri* group. At Bunsu plantation, where *Canthium* was artificially propagated 10-15 years ago, and stands some four to the acre, up to 40 per cent. of the cacao is infested with *africana*, to the virtual exclusion of other species; in areas of the plantation 150-200 yards away from the nearest *Canthium*, the ratio is more normal at one collection of *africana* to eight of *depressa*.

C. depressa and *buchneri* invariably build nests of tough vegetable carton in the canopy of cacao and various forest trees. In the latter case, the nests may be of very large size, and on the main trunk of the host tree. Wheeler (1922, Plate X) gives an excellent photograph of a large nest of *depressa* subsp. *fuscipectus*. The *depressa* group swarm in March and April in the Gold Coast, winged forms having been taken from nests examined on March 24th, 30th, and April 3rd and 5th, again more or less at the start of the wet season. Nests of the group have been taken on cacao (small in size, about 6 × 8 × 10 inches), and on *Pithecolobium saman*, *Cola cordifolia*, *Tetrapleura tetraptera*, *Peltophorum ferrugineum*, and *Euntumia elastica*. The group is essentially African, and members have been taken in the Ivory Coast, Gold Coast, Nigeria, the Congo, and (by Weber, 1943) as far east as the Imatong mountains in the Sudan. The group as a whole is by no means strongly attracted to mealybugs. It appears to favour particularly the genera *Lecanium* and *Saissetia*, over which it builds carton tents thickly interlaced with fungal debris, in such a way that they appear remarkably like bird droppings at a distance. The tents are usually small in size (about $\frac{1}{2}$ an inch in diameter), and contain few Coccids, though often of several different genera. For example, a tent recently examined by the writer at Oyoko contained three adult specimens of a *Lecanium*, one adult *Ceroplastes*, and one nymph of *Paraputo ritchiei*.

C. africana is an important species because of its close association with *P. njalensis* in *Canthium*. As with the *depressa* group, it tends to build relatively small nests (about 400-500 cu. inches) in the canopy of cacao trees, but very large nests (3 feet long, 1 foot wide, 10 to 12 inches deep at the centre) on the trunk of *Canthium*, often with small subsidiary nests higher or lower on the trunk. In one *Canthium* tree felled

and dissected in its entirety, the base of the main nest was situated 20 feet above ground level on the main trunk, with a subsidiary nest at 18 feet. The trunk of the tree was relatively smooth up to the 15 foot level, when it became possible to discern the nodes. No entrance holes to trunk domatia were apparent below, and the trunk was solid up to the 18 foot level.

Gravid queens were not found in either of the carton nests on the trunk. Dissection of the stem, however, showed that the colony queen was kept in a domatium in one of the main trunk nodes, close to the main nest. In a second dissected tree, the queen was found inside node 21, which was partly encircled by two nests at the 13 foot level. Though, in this case, the main nest was clearly at the two foot level, there were no stem domatia at this point, nor were there any until node 21 at the 13 foot level.

C. africana carton nests have been taken from *Ficus asperifolia*, *Terminalia superba*, *Rauwolfia vomitoria*, *Pseudospondias microcarpa*, *Cola cordifolia*, *Dis-coglyprena caloneura*, *Mitragyna* sp., *Pithecolobium saman*, *Antiaris africana*, *Erythrina altissima*, *Citrus* sp., *Piptadenia africana*, and *Musanga smithii*, in addition to cacao and *Canthium*.

C. africana is widely distributed throughout West and Central Africa, and it is undoubtedly an indigenous species. Winged forms have been taken in nests dissected in March, April, and May, the earliest being 17th March, the latest 18th May. As in the case of the other Crematogasterine ants, it swarms early in the wet season.

C. africana is rarely associated with Coccids other than mealybugs (according to Bailey in Wheeler (1922) it is associated with *P. crassipes* Newst. in domatia in *Sarcocephalus* sp. in the Congo), but on several occasions specimens of a species of *Saissetia* have been taken from the tops of *Canthium* trees in and around Tafo which were being sporadically attended by *C. africana* nesting in the trees.

The other ants in the *Atopogyne* group are relatively rare (52 random collections to 27 of *africana* on cacao). All are arboreal, although they differ from the species already discussed, particularly in their nest building habits. *C. cuvierae* has been taken only once, at Asuansi, in the Western Province of the Gold Coast. *C. stadelmanni* seems to be the commonest of the minor species. It has been taken on *Ricinodendron africana*, *Loranthus* sp., *Terminalia superba*, *Mimusops elengi* (nest under bark), and *Aleurites montana*. Winged forms have been taken on two occasions, once in May, and once in November. The species is particularly commonly associated with *Farinococcus* and makes its nests in *Loranthus* cankers.

(3). *Crematogaster* group. One species only, *C. sp.* F.257, has to be considered and this is actually the second dominant Crematogasterine cacao ant (448 collections to 778 of *striatula*). Nests are very similar to those of the *Sphaerocrema* group—small, under flakes of bark, with little carton. Swarming takes place from March to May (earliest date noticed 24th March, latest 5th May). This species has been taken only once on an indigenous tree, when a small nest was found on a branch of a *Ficus* sp.

(4). Other Crematogasterine species. This consists of a group of five species all rare and of very minor importance. No information is available on habits. The species of *Orthocrema* was first taken attending nymphs of an undetermined *Steato-coccus* sp., on *Tetrapleura tetraptera*, on which the ants had a small carton nest. It has been taken subsequently in Togoland attending an undetermined species of *Pseudococcus* on cacao, in association with *Atopomyrmex mocquersyi* on *Erythrina* sp. at Tafo, attending species of *Lecanium*, attending *Paraputo ritchiei* on *Triplochiton scleroxylon* and nine times associated with mealybugs on cacao. The species of *Deca-crema* has only been taken in association with mealybugs on cacao at Tafo (eight times).

(b). *Pheidolini*.

(1). *Pheidole megacephala*. In the present work it is certain that many of the numerous varieties, sub-species, and races of this cosmopolitan ant have been included under the single specific name. It is an important species, but the varieties that occur in association with cacao and mealybugs in the Gold Coast all appear to have very similar habits, and no useful purpose would be served by sub-division. The species is essentially ground nesting. This habit renders it of considerable importance as a mealybug tender on cacao seedlings that are too small to attract arboreal nesting species of *Crematogaster*, and it is also of importance in the case of mealybugs infesting the trunks, pods growing on trunks, and chupon growth, of mature cacao. On two occasions it has been taken nesting in a mature cacao tree, once in a dead branch in the canopy, and once in a squirrel-damaged pod five feet above ground level. Winged forms have been taken from soil nests on 22nd March, 1st May and 4th May, indicating that this ant normally swarms early in the wet season. Apart from *P. njalensis* on cacao, it has been taken in association with *P. citri* on *Ricinodendron*, on *Musa sapientum* attending *P. brevipes* (Ckll.) and on *Dioscorea* sp. with *P. citri*, in every case with soil nests at the base of the host plant. It has also been taken attacking mealybugs, and also carrying off and eating moribund nymphs of the Mirid, *Distantiella theobroma* (Dist.). It is by no means restricted to a coccidophilic habit.

(2). Other *Pheidole* species. The 10 other species of *Pheidole* taken in the present work are mostly soil species, and relatively rare: not one of them has been taken on more than three occasions and four of them only once. They have not been specifically determined, and since they are so rare they are regarded as of very minor importance. *P.sp.F.217* was first taken from a carton nest in a folded leaf of *Diospyros xanthochlamys* in forest at Bunsu on July 7th, two winged males being taken in the nest at the same time. *P.sp.F.530* has also been taken with an arboreal earthen nest on *Pseudospondias microcarpa*. *P.sp.F.522* was first taken from a small nest in a split in the bark of the trunk of a mature cacao tree on 24th March, when a gravid queen and brood were also secured. *P.sp.F.217* is the commonest of the 10 species, and has been taken most frequently in association with mealybugs on cacao pods at Tafo.

(c). Other *Myrmicinae*.

(1). *Macromischoides aculeatus*. This fierce tree dwelling ant is of considerable importance because it is negatively correlated with mealybugs and their attendant ant species. It invariably builds arboreal nests of leaves loosely woven together with pieces of vegetable debris, and fungal mycelium. Nests have been taken consisting solely of a carton tent fixed to the under surface of a single leaf on several occasions. The dimensions are about $3 \times 2 \times \frac{1}{2}$ ins. although larger nests are common, and often more than one nest will be found on the same tree. In the latter case, not every nest contains a queen and brood. *M. aculeatus* swarms at the start of the wet season, winged forms having been taken in nests in March and April. The species has been taken nesting on *Cola* sp., *Lonchocarpus*, *Creiba pentandra*, and *Musa sapientum*, apart from cacao, but does not appear to have any marked host preferences. Wheeler gives a photograph of two nests of this species. It is almost certainly a scavenger by habit, and its fierce nature is an indication that it also has predatory instincts.

(2). *Atopomyrmex mocquersyi*. Common locally in small areas of an acre or so, and very often associated with *Crematogaster* ants, although the precise nature of this association is obscure. It is very probably a mealybug rather than an ant association, since *Atopomyrmex* is avid in its attentions to mealybugs. It is a slow moving, sluggish, species that nests in the stumps of broken branches, and is invariably arboreal. According to Wheeler, it sucks nectar from an Anacardiaceous host, which confirms that it has a preference for food of a sweet nature. The relative abundance on cacao is 36 to 778 of *C. striatula*. It has been taken attending *P. njalensis* and an undetermined species of *Pseudococcus* in a mixed colony on *Distemonanthus benthamianus*, and also, with nest, on *Tetracarpidium conophorum*. It is

essentially a West and Central African species, although it also occurs in East and South Africa.

(3). *Meranoplus nanus*. This tiny ant has not been taken in the course of the quantitative work on which the relative densities of the various ant species have been computed. It has, however, been taken three times at Tafo, twice attending a mixed colony of Aphids (*Toxoptera coffeae* Nietner), and *P. njalensis* on cacao. The nest was in the soil at the base of the tree. The third time attending Aphids on young cacao pods. Wheeler (1922, page 184), cites Arnold as describing this species as mainly carnivorous.

(4). *Paedalgus termitolestes*. This minute ant is, as its name suggests, often associated with termites. It has been taken nine times on cacao, usually on the trunk close to the ground. Once it was taken in association with *P. njalensis*, and was undoubtedly palpating the mealybugs and receiving honeydew.

(5). *Catantolus parallelus*. This, and two other species of the genus, both probably new to science, are not uncommon on cacao, 60 random collections having been made. All are arboreal, nesting in hollow twigs or broken branches. Arnold states that they are predacious on termites. Nests have been taken on *Cola* sp., in the rotting trunk of a felled *Ceiba pentandra*, *Musanga smithii*, *Terminalia superba*, and *Alchornea cordifolia*. *C. parallelus* undoubtedly attends mealybugs sporadically, particularly *P. njalensis*, and was taken once in association with a colony of *F. virgata*. Winged forms have been taken in nests examined on 9th January, and 2nd and 11th May, indicating that swarming usually takes place early in the wet season.

(6). Other Myrmicine species. The species of *Tetramorium*, *Solenopsis*, and the unidentified species are all of very minor importance. *Tetramorium* is an Ethiopian genus, and the Gold Coast cacao species are all soil nesting. One of the species (F.541), is sporadically associated with mealybugs. The *Solenopsis* spp. are also soil nesting, and may be predacious on young mealybug nymphs.

(d). *Dolichoderinae*.

This group of species is more commonly associated with young seedling cacao than mature trees. They are essentially soil nesting. One species, *Technomyrmex detorquens* is cited by Le Pelley (1943) as an avid tender of *P. lilacinus* Ckll. on cacao at Peradeniya, in Ceylon.

(e). *Formicinae*.

(1). *Acantholepis*-*Nylanderia* group. Small black ants, essentially soil nesting, and rarely found on other than seedling cacao, where they are undoubtedly scavengers, though *Acantholepis* sp. F.517 has been taken from time to time milking *P. njalensis*. They are of little, if any, economic importance so far as cacao is concerned.

(2). *Oecophylla* group. There is only one species present, *O. longinoda*, although this is present in two forms, the typical form, and the variety *fusca* (Emery). There are no differences, as far as can be ascertained, in the habits of these two types, and they are henceforth dealt with collectively. As has been pointed out above, *Oecophylla* is the dominant cacao ant in the Gold Coast. It is of importance not only because of its evident pugnacious attitude towards mealybug tending species, and the cacao Mirids, but also because it is almost invariably associated with species of the genus *Stictococcus* which, in the Belgian Congo, are said to cause economic damage to cacao pods (Mayné & Ghesquière, 1934). So far as can be ascertained, these Coccids are not sufficiently abundant in the Gold Coast to cause any damage, but this does not necessarily mean that, with changing ecological conditions, they may not become pests in their own right at some future date. The leaf nests of this remarkable ant have been adequately described by Wheeler. They are invariably situated in the cacao canopy, and are less commonly found on young cacao. Winged forms are common, particularly in March and April, though once a freshly fertilised queen was

taken over a brood of 14 eggs on the underside of a cacao leaf in the middle of January. It appears that the young gravid females actually remain standing over their brood until the eggs have hatched. Generally speaking, where *Oecophylla* are present on a tree there is also a nest although, in a few of the 1,029 collections of this ant examined, it was clear that a single large nest was the base for colonisation of two or three adjacent cacao trees. Not uncommonly, several nests can be found on a single tree and this is particularly the case on *Citrus* sp. *Oecophylla* usually associated with Stictococcids, has been collected from *Harrogana madagascariensis*, *Coffea* sp., *Lonchocarpus* sp., *Cola cordifolia*, *Cola acuminata*, and a variety of undertermined bush plants and vines.

(3). *Camponotus* group. This group can again be split up into those species of arboreal habit, occurring on mature cacao (*C. chrysurus*), and soil nesting forms that occur on the trunks of mature trees as predators, or on seedling and young cacao up to 8-inch girth with soil nests, (*C.* spp. F.120 and 423). Of this group of species F.120 is usually associated with Aphids, and less commonly with species of *Stictococcus*. The other species is sporadically associated with Aphids and Coccids, but is mostly predacious and as such beneficial. Winged forms have been taken in nests in March and April.

(4). *Polyrhachis* group. The species of this group are essentially arboreal. *P. militaris* and *laboriosa* nest usually in trees, although at times they may be found in rotting branches on the ground. The tree nests are of a very loosely woven "fluffy" carton attached to several leaves. The other species are all arboreal, and build small circular carton nests on the undersides of leaves. *P. fissus* is the dominant species, *P. revoili* the second dominant. Both are common in relatively restricted areas, but rare elsewhere. Winged forms have been taken in nests as follows: *P. revoili*, 10th February, 5th May, 11th August, and 3rd December; *P. fissus*, 29th May and 20th December, and *P.* sp. F.487 on 28th January. *Phasmomyrmex polyrhachoides* which is closely related to *Polyrhachis*, has been taken only on cacao, on eight occasions: it is of very minor importance.

(f). *All other ants.*

The Ponerine, Doryline, and Pseudomyrmine species are all very rare on cacao, and are of insignificant importance. It is inevitable, for example, that one should find from time to time an occasional specimen of the common army ant, *Dorylus nigricans*, scavenging on the trunk of a cacao tree, and the same applies to *Platythyrea conradti* which, however, is generally distributed and fairly common on young cacao. All are essentially soil dwellers, predacious, and hence beneficial. They have not been taken, apart from predatory activities, in association with mealybugs.

Summary.

The entomology of swollen shoot of cacao is a complex and unique problem involving the inter-relations in the field of over 120 insect species of four insect and two arachnoid orders. Briefly, there are 17 species of pseudococcids, 75 species of ants, 16 species of hymenopterous parasites, three predatory beetles, one predatory dipteran, and three arachnid species involved in vector relationships directly, with a further 18 Coccid species involved indirectly (it is possible, of course, that further work will show that some of these 18 species are directly concerned as vectors).

In the present paper an attempt has been made to reduce this assemblage of insect material to some semblance of order. The Coccid species are named and a series of preliminary observations on their biology and field behaviour detailed. The ant species, some of which are obligatorily associated with certain vector species, have been sorted into groups where specific determination has proved impossible or unnecessary and information has been included on their field habits and relative abundance.

There are three distinct but complementary ecological niches involved in the problem. The first, and most important, is the association between the mealybug virus vectors and the Myrmicine coccid-tending ants. The second is the association between mealybugs of the genera *Paraputo* and *Formicococcus* and the wild forest tree hosts of swollen shoot virus, and the third is the negative association between the mealybug tending Myrmicine ants and *Oecophylla* and *Macromischoides*, the latter species acting in certain circumstances as barriers to the spread of the mealybug-tending species and hence to the spread of mealybugs and virus. These problems will be dealt with on a quantitative basis in a further paper.

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References.

- ANON. (1948). Rep. W.Afr. Cacao Res. Inst., 1947-48.
 BÜNZLI, G. H. (1935). Mitt. schweiz. ent. Ges., **16**, pp. 455-593.
 CARTER, W. (1939). J. Anim. Ecol., **8**, pp. 261-276.
 HALL, W. J. (1945). Bull. ent. Res., **36**, pp. 305-313.
 JAMES, H. C. (1937). Bull. ent. Res., **28**, pp. 429-461.
 LE PELLEY, R. H. (1943). Trans. R. ent. Soc. Lond., **93**, pp. 73-93.
 MAYNÉ, R. & GHESQUIÈRE, J. (1934). Ann. Gembloux, **40**, pp. 3-41.
 POSNETTE, A. F. & STRICKLAND, A. H. (1948). Ann. appl. Biol., **35**, pp. 53-63.
 POSNETTE, A. F. & STRICKLAND, A. H. (1949). Nature, **163**, pp. 105-106.
 SANTSCHI, F. (1918). Bull. Soc. ent. Fr., **1918**, pp. 182-185.
 STRICKLAND, A. H. (1947). Bull. ent. Res., **38**, pp. 497-523.
 STRICKLAND, A. H. (1950). Proc. R. ent. Soc. Lond., (A) **25**, pp. 1-9.
 WEBER, N. A. (1943). Bull. Mus. comp. Zool. Harvard, **93**, pp. 261-389.
 WEBER, N. A. (1944). Ann. ent. Soc. Amer., **37**, pp. 89-122.
 WHEELER, W. M. (1906). Bull. Amer. Mus. nat. Hist., **22**, pp. 1-18.
 WHEELER, W. M. (1922). Bull. Amer. Mus. nat. Hist., **45**, pp. 1-1,139.
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A LIFE-HISTORY STUDY OF *ENDROSIS LACTELLA* (SCHIFF.) (LEP. OECOPHORIDAE).

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(Pls. XIX—XXI.)

The White-shouldered House Moth, *Endrosis lactella* (Schiff.) (= *Endrosis sarcitrella* (L.)) is widely distributed in Britain. It is frequently found associated with the Brown House Moth, *Hofmannophila pseudospretella* (Staint.) although it is less common in private houses than the latter species. O'Farrell & Butler (1948) describe it, with *H. pseudospretella*, as the commonest warehouse moth in Northern Ireland during the period 1942-1946, and are of the opinion that it is more a stored products pest and less of an omnivorous scavenger than the Brown House Moth. It rarely attacks clothing, and this probably accounts for the fact that it occurs in dwelling-houses less commonly than *H. pseudospretella*. Major infestations occur more frequently on stored seeds, such as peas and beans, than on other products. In addition to its usual indoor habitats, it occurs in the open air and has been reported breeding in birds' nests (Waters, 1929). The field distribution and preferred habitats of *E. lactella* correspond closely with those of *H. pseudospretella*, but it will be seen that, in most details of its life-history, it differs considerably from that species, the apparent similarity in habits of the two being due to common humidity requirements and similar food preferences.

Most of the literature quoted in connection with *H. pseudospretella* (Woodroffe, 1951) deals also with *E. lactella*. Austen & Hughes (1948) give a useful general account of the species, and the taxonomy is dealt with by Corbet & Tams (1943) for adults, Hinton (1943a) for larvae, and Hinton & Greenslade (1943) for pupae. Hinton (1943c) gives the characters distinguishing the larva from that of *H. pseudospretella*. Materials recorded as being attacked include cocoa (Andres, 1920), carpets (Laing, 1932), bird guano (Hinton & Greenslade, 1943), stored cereals and dried fruit (Corbet & Tams, 1943), insects in or near spiders' webs (Hinton, 1943b), keratin fibres (Goodall & others, 1946), and bulk wheat (Richards & Waloff, 1947).

Throughout this work a continuous supply of insects was obtained from a single mass culture. This consisted of a metal bin of dried grass which had been stored for several years in an unheated outhouse, and had been completely undisturbed. It was heavily infested with *H. pseudospretella*, *E. lactella*, and a few *Borkhausenia fuscescens* (Haw.), and was maintained as a culture by storage in an underground concrete shelter where the relative humidity was normally between 80 per cent. and 90 per cent. The lid was removed and the bin covered with muslin in order to prevent escape of the moths. (See also Woodroffe, 1951.)

The Egg.

The eggs of *E. lactella* are dull white in colour, without gloss, and with no visible surface pattern; they are long and narrow, and taper conspicuously towards one end. They are very soft when first laid, and adhere strongly to the surface upon which they are deposited. Frequently they stick together, and are found as rows, plates or masses of eggs (see Plate XIX, fig. 1). It is usually impossible to separate the individual eggs of such a group, or to detach the mass as a whole from the surface to which it is adhering, without damaging a high proportion of the eggs. They are deformed by gentle pressure by a camel-hair brush when first laid, and, although they harden to some extent after a few days, they remain very difficult to handle. The thinness of the shell can best be appreciated if eggs are examined just before they hatch. The fully-developed embryos appear quite naked, and only their curled-up position indicates that they are still within the egg-shell.

The fully-extended ovipositor of the female is as long as the abdomen, and eggs are almost invariably laid deep in some crevice or between the fibres of some coarse material, where they are deformed by pressure and often bear the imprint of the surfaces with which they have been in contact.

Measurements were made with a micrometer on a group of 20 eggs which had not been deformed, and groups of 50 eggs were weighed. The mean egg length was .55 mm. (.52-.58 mm.), the mean breadth was .28 mm. (.26-.29 mm.) and the mean weight of 8 groups of 50 eggs was 1.0 mg., giving an average weight per egg of .02 mg.

The Incubation Period.

The length of the incubation period was determined over a range of conditions which were controlled by the use of incubators, potash solutions and sulphuric acid as has been described for *H. pseudospretella* (Woodroffe, 1951). All eggs were laid at 70 per cent. R.H. and 25°C. and the time of laying was known to the nearest half-day. Large sheets or masses of eggs were not used because of the inevitable destruction of unhatched eggs by newly-hatched larvae under these conditions. The eggs were incubated in groups of 20 in 2 inch \times $\frac{1}{2}$ inch tubes, and young larvae were removed as soon as possible after emergence. Counts of larvae were made every half-day, and the incubation period for each set of conditions was determined as the mean for 60 eggs. Hatching usually occurred over a period of about 24 hours, but variation between individual eggs was slightly greater at the lower temperatures. The results are set out in Table I (see also fig. 1). It will be seen that the eggs are sensitive to temperature, but comparatively insensitive to humidity.

TABLE I.

Mean incubation period in days of sets of 60 eggs under various physical conditions.

R.H. %	TEMPERATURE °C.									
	10	13	15	20	25	26	27	28	29	30
90	42 (39-44.5)	22.7 (22-24) 22.4 (21.5-24)	15.1	8.6	6.3	6.0	7.2	6.7	6.8	Failed to Hatch
30	—	—	16.1	9.7	7.2	7.5	7.7	7.0	—	—
8.5	—	—	16.8	—	7.8	—	—	—	—	—
3.2	—	—	16.8	11.7	8.0	—	—	—	—	—

Percentage Survival.

The proportion of larvae emerging successfully from eggs incubated under various conditions was determined by a separate series of experiments. It must be remembered that these eggs were selected single eggs of typical shape, and so did not constitute a representative sample of the total egg output of the females from which they were obtained. Three groups of 20 eggs were used for each set of conditions, and the results are given in Table II.

TABLE II.

Percentage survival of eggs (sets of 60) incubated under various physical conditions.

R.H. %	TEMPERATURE °C.							
	10	13	15	25	27	28	29	30
95	—	—	61	77	—	—	—	0
70	73	65	80	83	75	26	0-5	0
45	—	—	55	42	33	16	0	0
8.5	—	—	—	10-30	—	—	—	—
approx. 1	—	—	—	0-20	—	—	—	—

The proportion of eggs showing no sign of embryonic development under favourable conditions was approximately 10 per cent. The high mortality at the lowest humidities and the highest temperature was measured 5-8 times, and the survival varied as shown by the ranges given in the table.

It proved extremely difficult to obtain reliable figures for survival from complete batches of eggs. Apart from the practical difficulty of collecting all the eggs laid by a single female without damaging a proportion of them, it was necessary to incubate the eggs as they were laid - in sheets and masses. There was always a tendency for newly-emerged larvae to eat the remaining unhatched eggs, and if some of the eggs in the centre of a mass hatched early, the larvae had no alternative but to eat their way out. The following figures, therefore, concern the survival that may, in practice be expected, but do not indicate the proportion of non-viable eggs normally laid by a female. For 16 complete batches of eggs, laid and incubated at 70 per cent. R.H. and 25°C., survival varied between 8.5 per cent. and 81.0 per cent., with a mean value of 44.1 per cent.

The Larva.

Comparison with Larva of Hofmannophila pseudospretella.

Hinton (1943c) has described the features which may be used to distinguish the larva of *E. lactella* from that of *H. pseudospretella*. It is frequently necessary to be able to distinguish the two species in practice because of their similar field distribution. Hinton's separation is based on the structure of the adfrontals, the position of the frontal puncture, the number of ocelli, the structure of the labium, the shape of the eighth spiracle and the position of certain Pi-group setae. All these features are difficult to see, particularly if the larvae are small. Some, such as the number of ocelli and the shape of the eighth spiracle, are variable, and the determination of setal differences is a matter requiring considerable practice. There are, however, several differences which have not been emphasised elsewhere, but which are, in practice, extremely useful for separating the larvae of the two species.

(a). The general body colour in *H. pseudospretella* is white and uniformly glossy. The colour in *E. lactella* is dull white, with rows of shiny spots representing the chitin plates at the bases of the setae. Using a binocular microscope, a black background and suitably adjusted lighting, quite small larvae may readily be identified by this method (see Pl. XIX, fig. 2 and Pl. XX, fig. 1).

(b). In *H. pseudospretella* the prothoracic shield is broad, indefinite in outline and pale amber in colour. In *E. lactella* the shield is narrow, clearly outlined and definitely brownish, especially laterally (see Pl. XX, fig. 1).

(c). The appearance of full-grown, wandering larvae on sacks or walls is often the first visible sign of an infestation. The two species may easily be separated on account of size differences. Larvae of *E. lactella* seldom exceed 14 mm. in length, and usually weigh between 10 and 20 mg. Larvae of *H. pseudospretella* seldom measure less than 16 mm. and usually weigh between 50 and 100 mg.

Some larvae of both species secrete a fluid from the head region when roughly handled. This fluid is often pale amber in *H. pseudospretella* and dark brown in *E. lactella*, but the difference is not constant.

Although superficially similar in appearance, the cocoons of the two species differ in texture. This difference will be described in the section on the pupa.

Development upon middlings under controlled conditions.

Larvae were reared on partly sterilised middlings under a variety of conditions, and measurements were made of the length of the growing period and the number and duration of the instars. The young larvae were confined in 2 inch \times $\frac{1}{2}$ inch

tubes in groups of 10, and subsequently transferred to 3 inch \times 1 inch tubes. Ordinary perforated corks and muslin were used to close the tubes, and no larvae succeeded in eating their way out (in contrast to those of *Hofmannophila*). Tubes of silk were constructed within the food mass and the larvae fed and moulted within these tubes. Rolls of corrugated paper were provided for pupation, and full-grown larvae often spun up without any obvious wandering period. In the absence of corrugated paper, the larvae readily spun up in the uppermost layer of food. The results, shown in Table III, give the mean duration of the growing period of about 20 larvae for each set of conditions (see also fig. 1).

At 70 per cent. R.H. and 15°C. a few small larvae were still alive after 80 days, but all had died by 130 days. At 30°C., larvae at 70 per cent. and 90 per cent. R.H. were all dead after 6 days.

TABLE III.

Mean growing period in days of larvae reared on middlings under various physical conditions.

R.H. %	TEMPERATURE °C.				
	10	13	15	20	25
90	133 (123-141)	102 (94-109)	73 (68-77)	42 (39-45) 57 (54-62)	38 (36-41) 49 (46-52)
80	—	—	—	—	—
70	—	NO LARVAE REACHED MATURITY			

Observation of the larvae growing at 90 per cent. R.H. and 25°C. and collection of head capsules showed that there were usually seven instars, the seventh moult occurring at pupation. An indication of the duration of each instar was gained by rearing 4 isolated larvae upon wool and yeast at 90 per cent. R.H. and 25°C. Development was slower than upon middlings but there were 7 instars, and the dates of moulting could be accurately noted. The mean duration of the instars of the four larvae was:—

INSTAR NUMBER	..	1	2	3	4	5	6	7
DURATION IN DAYS		5	5	6	5	10	9	10

Larvae reared at 70 per cent. R.H. moulted repeatedly without any appreciable increase in size.

Development upon various foods under controlled conditions.

Larvae were reared on a variety of foodstuffs at 90 per cent. R.H. and 20°C. Each food was tested with 20 newly-hatched larvae, in two 3 inch \times 1 inch tubes of 10 each. The food was in slight excess, and to it was added a fungicide ($\frac{1}{2}$ per cent. by weight of sodium orthophenylphenate, B.D.H. Reagent), but experience with *H. pseudospretella* (Woodroffe, 1951) had shown that this substance was not completely effective in controlling mould growth. The date of wandering of the first larva in each tube was taken as the date of completion of larval development. There was seldom more than a few days difference between the time that the first and last larva began to wander. Rolls of corrugated paper were provided for pupation, and the number of adults that finally emerged was noted. Table IV gives the growing period in days and the proportion reaching the adult stage for each foodstuff. In two cases, 5 per cent. by weight of dried brewers' yeast (Glaxo, debittered) was mixed with the food to supply vitamins.

It will be seen that the most rapid development occurred when the larvae were reared upon dead adults, and the slowest development was upon macaroni. The most satisfactory survival was upon whole wheat and the least satisfactory upon groundnuts. It is interesting that the highest survival recorded was only 75 per cent. The mortality occurred chiefly in the young larval stage.

TABLE IV.

Growing period and survival to adult stage of larvae reared on various foodstuffs at 90% R.H. and 20°C.

Foodstuff	Growing Period in days	Survival to Adults %	Remarks
Whole Wheat	47	75	Some damaged material present.
Middlings	46	70	
Dried Grass	54	70	Prepared commercially.
Beans	86	65	Some damaged material present.
Macaroni	109	65	
Dead Moths	40	60	Adults of <i>Hofmannophila</i> and <i>Endrosis</i> .
White Flour	77	60	
Wool and Yeast	67	60	Fine woollen cloth.
Leather and Yeast	93	35	Fine shreds and small pieces.
Groundnuts	104	20	Decorticated and broken.

It was shown that growing larvae of *E. lactella* were not adversely affected by a complete change in diet under constant conditions. Larvae were reared on whole wheat at 90 per cent. R.H. and 25°C. for 20 days, and half were then transferred to wool and yeast, also at 90 per cent. R.H. and 25°C. Similarly, larvae were started upon wool and yeast and half were later transferred to whole wheat. Development of the transferred groups was somewhat more rapid than that of the larvae which remained upon wool and yeast throughout.

There was no diapause in *E. lactella*. The full-grown larvae wandered for a short time, spun cocoons, and pupated within a fortnight (at 25°C.). The four or five days immediately preceding pupation were passed as a prepupa (see Pl. XX, fig. 2).

The Pupa.

The larvae of both house moths incorporate debris of various kinds into their cocoons, and it is impossible to separate cocoons of the two species on the basis of external appearance; but there is a difference in texture which can be used to differentiate between them. This difference can best be appreciated if the cocoons are torn open with a dissecting needle. The cocoons of both species are very tough, but that of *H. pseudospretella* is brittle, and tears like strong brown paper, and the pupa may be seen lying comparatively loosely inside. In contrast, the cocoon of *E. lactella* tears like cotton-wool, and the pupa is found to be closely invested by the innermost layers. These inner layers are pure silk and are responsible for the difference in texture between the two species.

The pupa (Pl. XX, fig. 2) is soft and white when first formed, but it rapidly hardens and becomes amber in colour. The eyes become pigmented, and the wing-sheaths darken immediately prior to emergence.

Pupae for the determination of the duration of the pupal stage were obtained by allowing wandering larvae to spin in a roll of corrugated paper. This was incubated for 10 days at 90 per cent. R.H. and 25°C. and then torn open. Freshly formed

pupae were used in the experiments, and prepupae were also collected, and used later as soon as they had pupated. Pupae were incubated in groups of 20 under various physical conditions, and the mean duration in days is given in Table V. The effect of temperature at 90 per cent. R.H. is illustrated in fig. 1.

TABLE V.

Mean duration in days of pupal stage under various physical conditions.

R.H. %	TEMPERATURE °C.						
	10	13	15	20	25	29	30
90	58.2 (50.5-81)	30.8 (27-35.5)	25.0 (24-27.5)	15.1	10.4	7.4	Failed to emerge
50	—	—	—	15.7	10.1	—	
15	—	—	—	15.9	10.7	—	

All the pupae at 29°C. and 90 per cent. R.H. began to hatch but only four succeeded in freeing themselves completely from the pupal skin and these died before the wings had expanded.

Total Development Period.

The time required for complete development from egg to adult at various temperatures was compiled from information presented in the preceding sections with the addition of the period between the spinning-up of the larva and the formation of the pupa. This period is approximately 7 days at 25°C., 10 days at 20°C., 12 days at 15°C., 18 days at 13°C. and 22 days at 10°C., giving total developmental periods of 62, 75, 135, 188 and 235 days respectively. The effect of temperature on the duration of the total development period is illustrated in fig. 1.

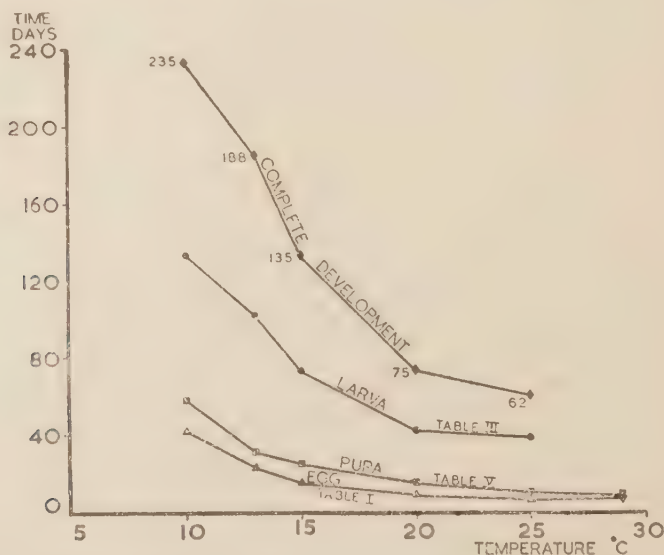


Fig. 1.—The effect of temperature on the duration of the total developmental period and of the egg, larval and pupal stages at 90% R.H.

The Adult.

Sex Ratio.

From time to time numbers of adults were taken from the bin of dried grass (see page 749) for various purposes, and, where possible, the numbers of the sexes in each batch were recorded. Further collections were made specifically for the purpose of determining the sex ratio and, in all, 26 counts were made over a period of 10 months, and a total of 3,417 adults were sexed. The highest male ratio recorded for a single collection was 1 male to 6 females, and on several occasions counts were made of 1 to 50. The total of 3,417 adults comprised 142 males and 3,275 females, a ratio of approximately 1 to 23.

Copulation and oviposition.

The study of oviposition in *E. lactella* proved to be the most difficult single investigation carried out in connection with this species. The laying habits of the female and the physical properties of the eggs which were responsible for some of these difficulties have already been described on page 749. The shortage of males mentioned above was intensified by a reluctance to mate on the part of the few males that were available. Finally, it was difficult to persuade the females to lay in such a manner that the eggs could be counted.

When pairs of unmated adults were confined in 3 inch \times 1 inch tubes mating was rarely observed, and viable eggs were obtained from only one pair in ten. Eggs laid on grain were deposited in layers deep in crevices and were impossible to count. Few eggs were laid in empty tubes except between the cork and the glass, and these were destroyed when the cork was removed. Many variations were tried out, and the following method was finally adopted.

Pupae were collected from the bin of dried grass, or from sub-cultures, and isolated in 2 inch \times $\frac{1}{2}$ inch tubes. The adults which had emerged were collected each day and placed in a large glass jar covered with muslin. Additional males were obtained whenever available and added to the jar. After 24 hours the females were removed one by one, weighed, and placed in 2 inch \times $\frac{1}{2}$ inch tubes. Each tube contained a strip of muslin about 6 inches long and $\frac{1}{2}$ inch wide, folded so as to form a pad about $\frac{1}{2}$ inch square. This was placed in the tube so that the open edges of the folds faced the top and bottom of the tube, thus presenting a series of deep crevices of varying widths to the ovipositing female. Eggs were deposited in these crevices, usually in the form of plates consisting of a single layer of from 10 to 50 eggs, adhering strongly to the muslin. After about 5 days (longer at the lower temperatures) or when the female was dead, the muslin strip was removed and unfolded, and the eggs deposited on it were counted under the binocular microscope. The dead female was dissected to determine whether fertilisation had occurred and, in doubtful cases, the eggs were also incubated. About one female in four produced fertile eggs or was found by dissection to have been fertilised, the proportion varying with the number of males available at the time. In no case was the bursa of a female found to contain more than one spermatophore. During the dissections it was noted that the ovaries of nearly every female examined contained large numbers of fully-developed eggs. This was found, not only in unfertilised females, but also in those which had died after an apparently normal period of oviposition.

Five experiments were carried out to determine to what extent egg production was influenced by physical conditions. In one experiment, drinking water was made available by placing small pieces of moistened filter paper against the glass inside each tube. This was subsequently moistened each day. Ovipositing females were frequently observed to drink from the pieces of filter paper but, obviously, the relative humidity could not be effectively controlled in this experiment.

TABLE VI.
Longevity and fecundity under various physical conditions.

Expt.	CONDITIONS		No. of ♀ Moths	Mean Wt. Mg.	Mean Eggs No.	Mean Eggs per Mg. Body Wt.	Mean Life. Days	Mean Life per Mg. Body Wt.	CORRELATION Values of r and p	
	R.H. %	Temp. °C.							Egg No. and Wt. of ♀	Length of Life and Wt. of ♀
1.	90%	25°	13	5.4	67 (32-122)	12.5	5.0 (1-8)	.94	r = .5587 p < .05 (Significant)	r = .6566 p < .02 (Significant)
2.	70%	25°	20	5.4	72 (14-145)	13.0	4.3 (1-10)	.80	r = .7658 p < .01 (Significant)	r = .7579 p < .01 (Significant)
3.	70% + Drinking Water	25°	23	6.2	122 (67-231)	19.9	8.9 (4-16)	1.51	r = .5354 p < .02 (Significant)	r = .1952 p > .1 Not Significant
4.	30%	25°	10	5.2	64 (19-134)	11.8	3.0	.61	r = .6489 p < .05 (Significant)	Not Significant
5.	90%	15°	21	6.4	106 (33-205)	16.0	9.1 (3-16)	1.58	r = .6737 p < .01 (Significant)	Not Significant
COMPARISON OF EXPERIMENTS									Nos. 2 and 3 With and without drink	
Significance of difference between mean egg numbers per mg. body weight					Nos. 1 and 4 Difference in R.H.		Nos. 1 and 5 Difference in Temp.		t = 3.845 p < .01 (Significant)	
Significance of difference between mean lengths of life per mg. body weight					t = .2928 p > .8 (Not Significant)		t = 3.615 p < .01 (Significant)		t = 4.840 p < .01 (Significant)	

The results of these five experiments are presented in Table VI and illustrated in fig. 2. The following points emerge from a study of the figures.

(a). In each case there was a significant correlation between the weight of a female at emergence and her egg production.

(b). At constant temperature, a difference in relative humidity did not give rise to a significant difference in egg output per mg. body weight (Comparison of Expts. 1, 2 and 4).

(c). At constant relative humidity, significantly fewer eggs were produced per mg. weight of female at 25°C. than at 15°C. (Comparison of Expts. 1 and 5).

(d). Under constant conditions, the provision of drinking water significantly increased the egg output per mg. body weight (Comparison of Expts. 2 and 3). In view of the result (b) above, it seems unlikely that the increased egg production was due to the inevitably higher relative humidity.

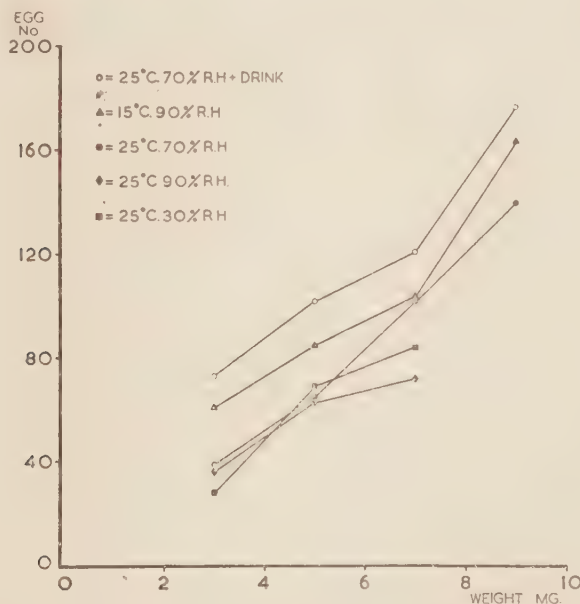


Fig. 2.—The relationship between egg production and weight at emergence of mated females.

The lightest female encountered in these experiments weighed 2.9 mg. and laid 61 eggs (at 90 per cent. R.H. 15°C.), and the heaviest weighed 9.4 mg. and laid 140 eggs (at 70 per cent. R.H. 25°C.). The lowest egg output recorded was 14 eggs produced by a female weighing 3.0 mg. (at 70 per cent. R.H. 25°C.) and the highest was 231 eggs by a female of 8.1 mg. body weight (at 70 per cent. R.H. 25°C. + drink). The lowest egg output per mg. body weight was 4.3 eggs (at 30 per cent. R.H. 25°C.) and the highest was 33.0 eggs (at 70 per cent. R.H. 25°C. + drink).

The oviposition of unmated adults was extremely irregular. Frequently no eggs were laid, or laying commenced only when the moth was dying. Dead, unmated females were often found with masses of eggs attached to the tips of their ovipositors. Occasionally a small number of non-viable eggs would be laid in a normal manner.

Longevity.

The oviposition experiments provided information about the length of life of mated females, and the figures are included in Table VI. In only two of the experiments could the longevity be correlated with the weight of the female at emergence, and, in Expt. 4., although the weights of the 10 individuals varied between 3.5 mg. and 7.6 mg., the length of life was 3 days in every case. It will be seen that longevity was significantly affected by temperature and by relative humidity, being shortest at high temperatures and low humidities. Life was prolonged by provision of drinking water to a greater extent than could be attributed to the high relative humidity.

One female lived for 16 days at 70 per cent. R.H. 25°C. + drink (weight 8.8 mg., eggs 130) and another (weight 7.3 mg., eggs 158) lived for the same period at 90 per cent. R.H. and 15°C. The shortest-lived female (weight 3.1 mg.) died after only 24 hours at 90 per cent. R.H. 25°C., during which time she laid 35 eggs.

The longevity of mated males could not be thoroughly investigated because so few specimens were available. They probably live up to 4 days at 70 per cent. R.H. 25°C., and up to 7 days at 90 per cent. R.H. 15°C. They seldom weighed more than 4.0 mg., and the lighter ones frequently died within 24 hours of mating. They were not observed to drink.

The length of life of virgin females was similar to that of mated females under comparable conditions, but tended to be slightly shorter. Unmated males lived considerably longer than mated males, a single specimen (weight 4.2 mg.) living for 26 days at 90 per cent. R.H. 15°C.

The considerable variation found in both longevity and fecundity is doubtless due to the fact that each affects the other, as well as being dependent upon the weight of the female. The effect of this third variable complicates the relationship between the other two.

Parasites and Predators.

No insect parasites appeared in any of the cultures of *E. lactella*, although a number have been reported attacking this species. Thompson (1945) lists four (*Angitia chrysosticta* (Gmel.), *Hemiteles bicolorinus* Grav., *Microbracon variegator* (Spin.), and *Trichogramma evanescens* (Westw.)). Richards (1949) records an additional Ichneumonid, *Phygadeuon bilinctus* (Gmel.).

The most important predator was the mite, *Cheyletus eruditus* (Schr.), which was observed to destroy considerable numbers of young larvae (see Pl. XXI, figs. 2 and 3). When a larva was attacked it reacted violently, and sometimes disturbed the mite sufficiently to escape. If, however, the mite was able to maintain its grip, the victim's struggles diminished rapidly, and it became motionless after about 10 seconds. If the mite was driven off as soon as larval movement had ceased, the larva remained motionless for some time (up to half-an-hour) but then recovered sufficiently to move about.

A gamasid mite, *Sciulus* sp., which was reported clinging to the bodies of living adults of *H. pseudopretella* (Woodroffe, 1951), also occurred in cultures of *E. lactella*. It was observed to eat the eggs of *Endrosis*, but was not found on the adult moths, and did not appear to attack the larvae.

Discussion.

Reference was made on page 749 to the opinion (O'Farrell & Butler, 1948) that *E. lactella* is more of a stored products pest and less of an omnivorous scavenger than *H. pseudopretella*. A study of the details of the life-histories of these two species as presented in this paper and previously (Woodroffe, 1951) reveals several reasons why this should be so. The larvae of both species require high humidity for satisfactory development. In Britain stored products do not normally meet this

requirement except for comparatively short periods, and ability to develop rapidly is therefore of primary importance. *E. lactella* is capable of very much more rapid development than *H. pseudospretella*. The larval growing period for the former is about 40 days at 90 per cent. R.H. 25°C., whereas, under these conditions, the larvae of the latter require 70 days, and then enter a period of diapause which may last another 160 days. The effect of this comparative rapidity of larval growth in *E. lactella* is reinforced by the short duration of the other developmental stages when compared with those of *H. pseudospretella*, and this advantage is maintained at low temperatures. The ability of a pest to develop reasonably rapidly at low temperatures is of immense importance when favourable humidity conditions are unlikely to prevail for long periods, and usually occur when temperatures are low. O'Farrell and Butler (1948) state that all stages of both species occur throughout the year in Northern Ireland, but Richards and Waloff (1947), reporting on the seasonal variation in numbers of insects in a London granary, observed adults only between May and October. In 1943, adults of *E. lactella* were observed a week earlier and a month later than adults of *H. pseudospretella*, and in 1944, the same observations were three weeks earlier and one week later. It is clear that, under field conditions, *E. lactella* can produce several generations a year, while *H. pseudospretella* normally completes only one developmental cycle in that time. There can, therefore, be little doubt that, under the conditions of storage that occur in this country, *E. lactella* is the more efficient stored products pest. It seems likely, however, that diapausing larvae of *H. pseudospretella* could survive conditions which would eliminate all stages of *E. lactella*.

Summary.

Endrosia lactella is widely distributed in Britain, where it is a minor pest of stored products, especially of grain and seeds.

The incubation period of the egg varied between 12 days at 10°C. and 6.0 days at 26°C. It was almost unaffected by changes in relative humidity.

Survival of eggs was low at high temperatures and low humidities, and the mean survival from complete batches of eggs at 70 per cent. R.H. 25°C. was 41 per cent. The highest survival recorded was 81 per cent.

On a diet of middlings the larval stage lasted 133 days at 90 per cent. R.H. 10°C., and 38 days at 90 per cent. R.H. 25°C. No adults were reared from larvae grown below 80 per cent. R.H. At 90 per cent. R.H. and 25°C. there were 7 instars.

On various foodstuffs at 90 per cent. R.H. 20°C., duration of the larval stage varied between 40 days on dead moths and 109 days on macaroni. Survival to the adult stage varied between 75 per cent. on whole wheat and 20 per cent. on ground-nuts.

The pupal incubation period was 58 days at 10°C. and 10.4 days at 25°C., and was approximately the same at all humidities.

The time required for complete development from egg to adult was 235 days at 10°C. and 62 days at 25°C. (90 per cent. R.H.).

There was a significant correlation between the weight of a female moth at emergence and the number of eggs laid. Egg output was lower at 25°C. than at 15°C., was not significantly affected by relative humidity, but was increased by provision of drinking water. Weights of females varied from 2.9 mg. to 9.4 mg. and egg output from 14 eggs to 231 eggs.

The sex ratio of the adults in the dried grass culture was found to be 1 male to 23 females.

Longevity could not be correlated with weight in all experiments, but, in the case of mated females, was dependent upon temperature, humidity and availability of drinking water. The mean adult life in days was 3.0 at 30 per cent. R.H. 25°C.;

5.0 at 90 per cent. R.H. 25°C.; 9.1 at 90 per cent. R.H. 15°C.; and 8.9 at 70 per cent. R.H. 25°C. + drink. Mated males were very short-lived (2–4 days at 70 per cent. R.H. 25°C.).

The only important predator was the mite, *Cheyletus cruditus*, which attacked the young larvae.

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References.

- ANDRES, A. (1920). Ueber den Messingkäfer (*Niptus hololeucus* Fald.)—Z. angew. Ent., **6**, pp. 406–407. (R.A.E., (A) **9**, p. 66.)
- AUSTEN, E. E. & HUGHES, A. W. MCK. (1948). Clothes Moths and House Moths.—Econ. Ser. Brit. Mus. (Nat. Hist.), no. **14**, 4th edn., 28 pp.
- CORBET, A. S. & TAMS, W. H. T. (1943). Keys for the identification of the Lepidoptera infesting stored food products.—Proc. zool. Soc. Lond., (B) **113**, pp. 55–148.
- GOODALL, F. L., GORTON, T. F. & SUMMERSGILL, J. V. (1946). DDT and its textile applications.—J. Soc. Dy. Col., **62**, pp. 189–198. (R.A.E., (A) **36**, pp. 323–324.)
- HINTON, H. E. (1943a). The larvae of the Lepidoptera associated with stored products.—Bull. ent. Res., **34**, pp. 163–212.
- HINTON, H. E. (1943b). House Moths (*Endrosis sarcitrella* and *Borkhausenia pseudospretella*) feeding on dead insects in or near spiders' webs.—Entomologist, **76**, pp. 4–5.
- HINTON, H. E. (1943c). Observations on species of Lepidoptera infesting stored products. III. Characters distinguishing the larvae of the House Moths, *Hofmannophila pseudospretella* (Staint.) and *Endrosis sarcitrella* (L.).—Entomologist, **76**, pp. 65–67.
- HINTON, H. E. & GREENSLADE, R. M. (1943). Observations on species of Lepidoptera infesting stored products. XI. Notes on some insects found in bird guano.—Entomologist, **76**, pp. 182–184.
- LAING, F. (1932). *Borkhausenia pseudospretella* and other House Moths.—Ent. mon. Mag., **68**, pp. 77–81.
- O'FARRELL, A. F. & BUTLER, P. M. (1948). Insects and Mites associated with the storage and manufacture of foodstuffs in Northern Ireland.—Econ. Proc. R. Dublin Soc., **3**, pp. 343–407.
- RICHARDS, O. W. (1949). Parasitic Hymenoptera found in British houses, warehouses and ships. I. Ichneumonidae.—Proc. R. ent. Soc. Lond., (B) **18**, pp. 19–35.
- RICHARDS, O. W. & WALOFF, N. (1947). Seasonal variations in the numbers of some warehouse insects.—Proc. R. ent. Soc. Lond., (A) **22**, pp. 30–33.
- THOMPSON, W. R. Ed. (1945). A catalogue of the parasites and predators of insect pests. Section I. Parasite Host Catalogue, Part 6. Parasites of the Lepidoptera (Ci-F). Belleville, Ont.
- WATERS, E. G. R. (1929). A list of the Microlepidoptera of the Oxford district.—Proc. Ashmol. nat. Hist. Soc., **1928**, 2nd pag., 72 pp.
- WOODROFFE, G. E. (1951). A life-history study of the Brown House Moth, *Hofmannophila pseudospretella* (Staint.) (Lep. Oecophoridae).—Bull. ent. Res., **41**, pp. 529–553.

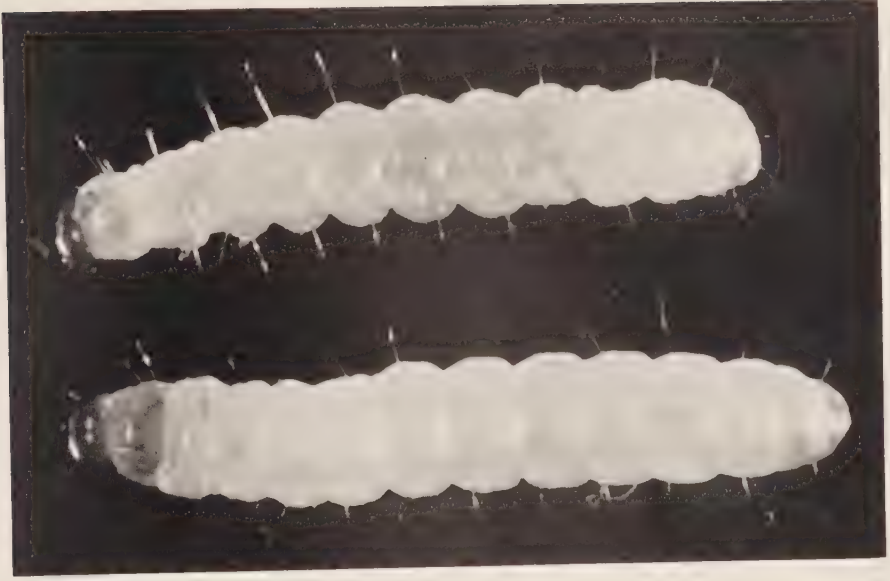


FIG. 2. Young larvae (4-5 mm.) of *E. lactella* (left) and *H. pseudospiretella* (right).



FIG. 1. *E. lactella*. Eggs and young larvae.



FIG. 1. Full-grown larvae of *E. lactella* (right) and *H. pseudospretella* (left).



FIG. 2. *E. lactella*. Prepupa (left) and pupae (right).

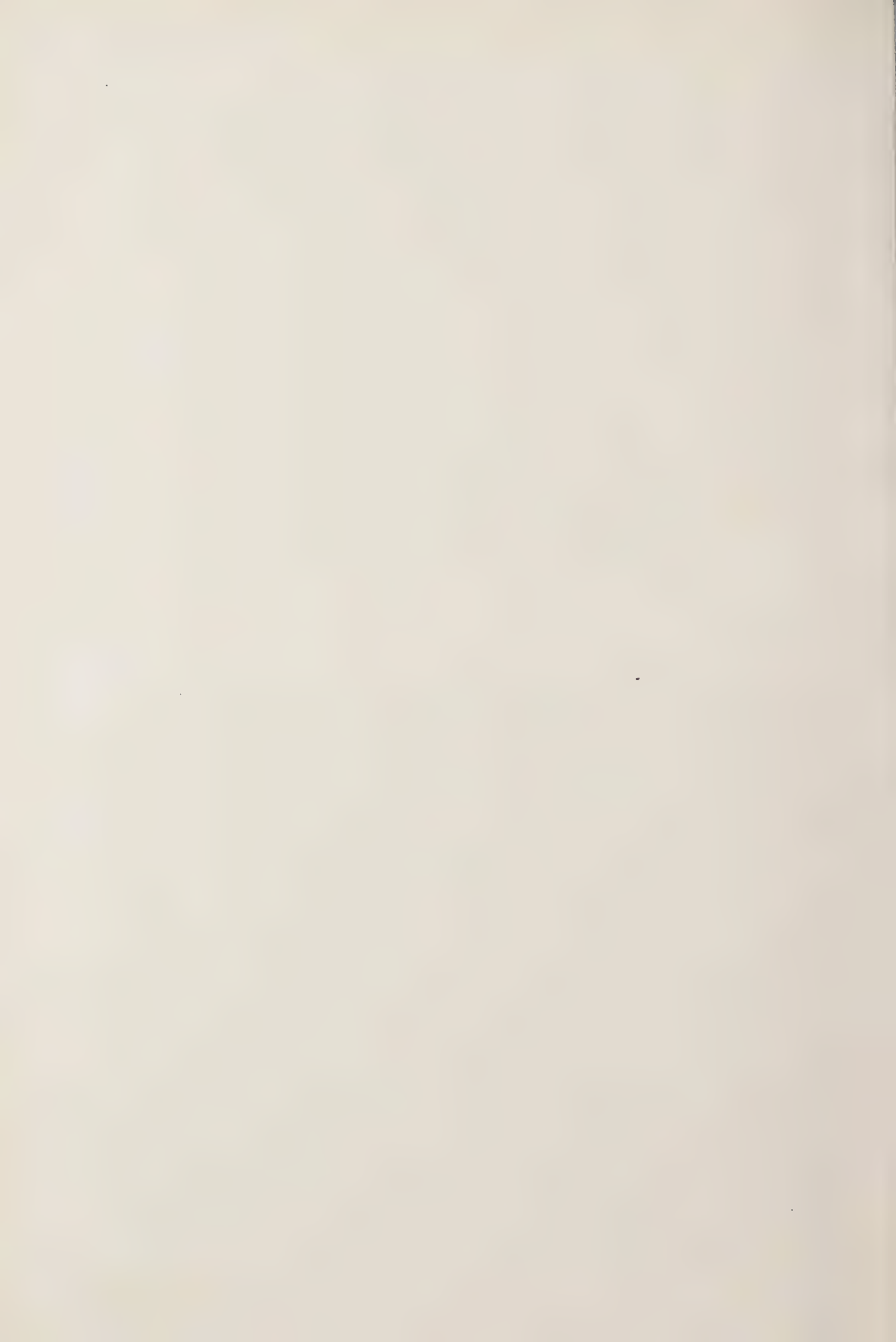




FIG. 1. *E. lactella*. Adults. Female (left) and male (right).



FIGS. 2 & 3. The predator, *Cheyletus eruditus*, attacking young larva.

A LABORATORY METHOD FOR TESTING RESIDUAL INSECTICIDES AGAINST ANOPHELINE MOSQUITOS.

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A. REACTION OF CAPTIVE MOSQUITOS TO GRAVITY AND LIGHT.

The experiments described in this section were performed in the course of developing a technique for laboratory testing of residual insecticides against Anopheline mosquitos. The object was to design a cage for the insecticide tests which would simulate natural conditions to some extent. In houses treated with residual insecticides, mosquitos can usually enter and leave freely by doors and windows, and it is known that DDT has an irritant effect on some species of *Anopheles*, causing them to leave rooms in which they would otherwise rest after biting. It was decided, therefore, to try to improve on the method generally employed in laboratory tests of residual insecticides, in which the mosquitos are introduced by hand into a treated room or cage from which there is no possibility of escape. The design, developed from the experiments, and the method of use, are illustrated and described below; the use of a light trap was suggested by the work of Muirhead Thomson with window traps (1948).

Description of Apparatus and Method of Use.

The apparatus (fig. 1) consists of a reservoir cage (R), a main cage (M) and a light trap (L). The reservoir cage is a box nine inches square with mosquito gauze sides and wooden top and bottom; there is the usual sleeve in one of the gauze sides. Pupae of *Anopheles* are placed in this cage to emerge, and the cage at this time is standing on a rack in an insectarium. The number of mosquitos emerging is estimated by removing and counting the pupal pelts. The adults are fed with water and split raisins, and just before an experiment is commenced any dead ones are removed and counted. In this way, all handling of the adult mosquitos is avoided, but the

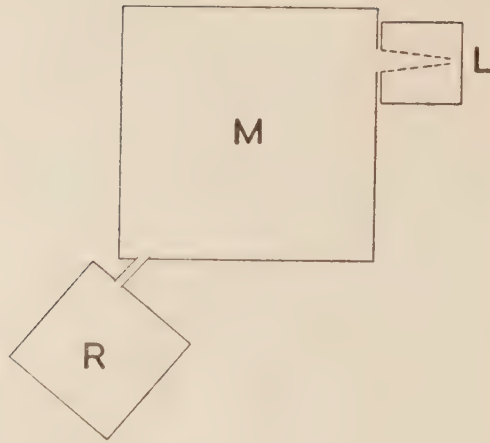


Fig. 1.—Diagrammatic side view of the apparatus or 'Cage' developed for testing residual insecticides against *Anopheline* mosquitos. R, reservoir cage; M, main cage; L, light trap.

number used in the experiment is known. In the top of the reservoir cage, close to one side, is a hole about half an inch in diameter, normally closed with a cork, but during an experiment the reservoir cage is connected to the main cage, by a glass or celluloid tube about three inches long which passes through this hole. The main cage is two feet tall with sides 18 inches wide, and consists of a wooden framework with three mosquito gauze sides, one of which is fitted with a sleeve. The fourth side, like the top and bottom, is of wood. This cage is designed so that plywood panels, sliding in grooves, can be fitted into the sides of the cage inside the mosquito gauze giving a dark closed box. Panels are provided for all sides except the floor, and the inner faces of the panels are treated with the insecticide under test. The light trap is a small box seven inches square which slides into position on the outside of the main cage. The top and the front are of mosquito gauze with a sleeve in the top, and the remaining sides are of light plywood. In the middle of the back is a circular hole two inches in diameter which coincides with a similar hole in the one wooden side of the main cage. The plywood panel for this side of the main cage has a similar hole. To this hole in the back of the light trap is fitted the base of a mosquito gauze cone about six inches long, the narrow opening of which (about half an inch in diameter) is held in place near the gauze front of the trap by means of cotton threads.

To perform an experiment, the reservoir cage containing about 50 mosquitos, male and female, is wrapped round with black cloth to exclude light, and then connected to the bottom of the main cage in the manner already described. This is done at 4 p.m. when the mosquitos average 36 hours old. About dusk (6–7 p.m.), the mosquitos become active and seek to escape by flying upwards, and in doing so they find their way up the tube into the main cage. By tilting the reservoir as illustrated, the mosquitos escape more readily since the mouth of the tube is then brought near the highest point of the reservoir.

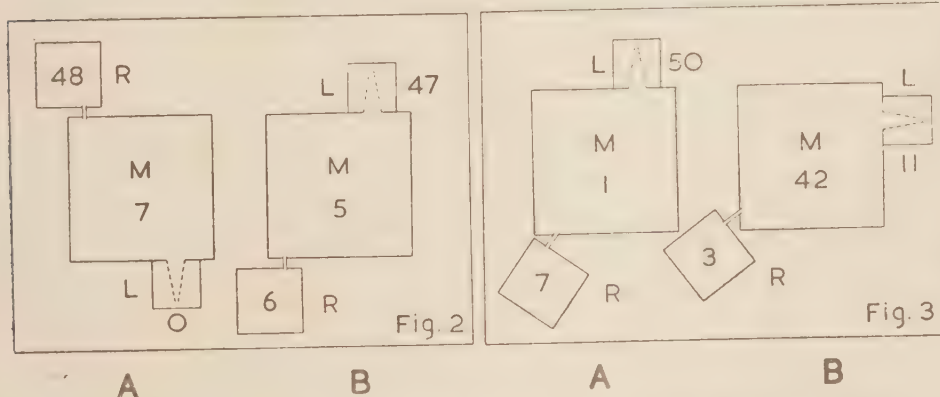
The light trap on the main cage points to a window through which the dim light of night enters, or else all shutters are closed and a dimmed low power electric light in the ceiling is used. The mosquitos are attracted to the dim light which enters the main cage through the light trap, and after resting on the panels of the main cage for varying and unknown lengths of time, they make their way through the gauze funnel and into the trap where they are found in the morning. The results of an experiment

with an insecticide are assessed by counting the numbers of living and dead mosquitos in the reservoir, main cage and light trap, and comparing these figures with those from an identical control cage with untreated panels. The experiment performs itself overnight.

Experiments.

The experiments which led to the design of cage, and method of use just described, were as follows. *Anopheles vagus* Dön., reared from wild caught larvae, were used because they could be obtained regularly in sufficient numbers, and development in this species is rapid. From the experiments which led to the development of the apparatus just described, it was concluded that under these conditions, *A. vagus* (♂ and ♀) showed clear cut reactions to gravity and light as described below.

Gravity. In complete darkness *A. vagus* adults had a strong tendency to fly vertically upwards. Figs. 2 and 3 illustrate the results of two experiments showing this.



Figs. 2 and 3.—To illustrate diagrammatically the arrangement of the cages, and the results of experiments on the behaviour of *A. vagus* in darkness. The line framing the cages in each figure indicates that the experiments were performed in a photographic dark room. The numerals show the distribution of the mosquitos at the conclusion of each experiment.

These two experiments were performed in a photographic dark room. Fig. 2A shows that when the reservoir was on top, nearly all the mosquitos remained in it, only 7 were found in the main cage, and none had entered the light trap which pointed vertically downward. In 2B where the positions of R and L were reversed, most of the mosquitos had found their way upwards out of R, through M and up into L. Fig. 3A repeats 2B, but in 3B, L was placed on the side of M, not on top, with the result that few mosquitos entered L, most remaining in M. In the experiment illustrated in fig. 3, the device of tilting R to facilitate the escape of the mosquitos, was adopted.

An experiment of two 'cages,' each set up in a manner similar to that illustrated in figs. 2B and 3A, with L on top of M, was performed prior to these, not using the dark room, but blacking out the reservoirs, tubes and light traps. Bringing the results of these experiments together, the distribution of mosquitos in the 4 'cages' in the morning may be summarised as in Table I. The term 'cage' is used here to denote the combination of reservoir, main cage and light trap.

TABLE I.

Experiments showing the upward flight of *A. vagus* in darkness. 'Cages' set up as in fig. 2B.

'Cage'	Reservoir (R)	Main Cage (M)	Light Trap (L)	
—	13	6	36	
—	6	2	46	%
Fig. 2, B*	6	5	47	enter-
Fig. 3, A	7	1	50	ing
				L.
TOTAL ..	32	14	179	79

* In this, and subsequent tables where the cage is specifically mentioned, the numbers against it will be found to correspond to those in the figure referred to.

An experiment was made in which one 'cage' was set up as in fig. 3B with L near the top of the side of M, not in the dark room, but with R and L blacked out. The results with these two 'cages' are shown below.

TABLE II.

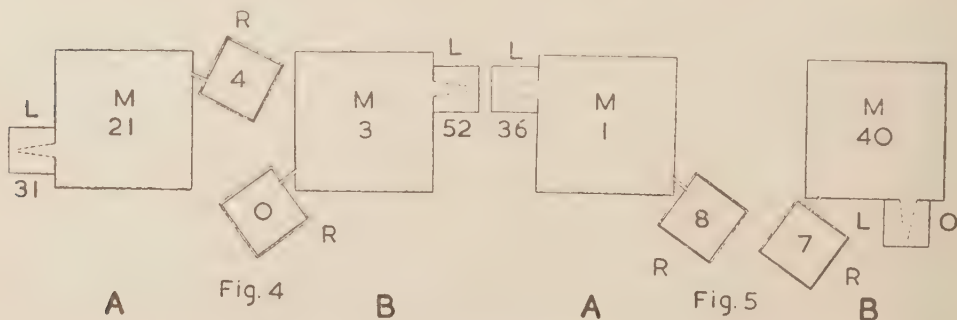
Experiments showing upward flight, and avoidance of lateral flight, by *A. vagus* in darkness. 'Cages' set up as in fig. 3B.

'Cage'	Reservoir (R)	Main Cage (M)	Light trap (L)	
—	1	53	1	%
Fig. 3B	3	42	11	entering
				L.
TOTAL ..	4	95	12	11

It is suggested that figures 2 and 3 and Tables I and II prove the strong tendency to upward flight in darkness, and avoidance of lateral or downward flight.

Light. *A. vagus* adults were attracted by a dim light which would deflect them from vertical upward flight to varying degrees, according to the position of the source of light, but would not induce them to fly vertically downwards.

Figures 4 and 5 illustrate the results of two experiments showing this:—



Figs. 4 and 5.—Illustrating the results of experiments to investigate attraction of *A. vagus* by dim light. The double line round R indicates that it was blacked out.

Figures 4B and 5A show that the majority of the mosquitos entered the light trap when it was on the side of the main cage near the top, though as fig. 3B and Table II show, very few will enter L in this position if it is in darkness. Figure 4A shows that if L is moved to the bottom of the side of M, the number of mosquitos entering L is reduced, a considerable proportion remaining in M; while if L is placed on the bottom of M (fig. 5B), all those which enter M remain there and none enter L.

In four other experiments, 'cages' were set up as illustrated in 4B and 5A with L towards the top of the side of M. This was the position finally adopted for the insecticide trials (see fig. 1). The results with the six 'cages' are shown in Table III.

TABLE III.

Experiments showing attraction of *A. vagus* by dim light when L is at the top of one side of M. 'Cages' set up as in fig. 4B.

'Cage'	Reservoir (R)	Main Cage (M)	Light trap (L)	
—	1	3	53	% entering L.
—	3	4	44	
—	2	7	44	
—	4	0	53	
Fig. 4B	0	3	52	
Fig. 5A	8	1	36	
TOTAL ..	18	18	282	89

In two other experiments 'cages' were set up as illustrated in fig. 4A with L towards the bottom of the side of M, except that R was connected at the bottom of the opposite side, instead of near the top. The results with all three 'cages' are shown in Table IV.

TABLE IV.

Experiments showing reduced attraction of *A. vagus* by dim light when L is moved to the bottom of one side of M. 'Cages' set up as in fig. 4A.

'Cage'	Reservoir (R)	Main Cage (M)	Light Trap (L)	
—	2	21	34	% entering L.
—	2	4	45	
Fig. 4A	4	21	31	
TOTAL ..	8	46	110	67

In one other experiment a 'cage' was set up as illustrated in fig. 5B with L on the bottom of M. The results from the two 'cages' are shown in Table V.

TABLE V.

Experiments showing that a dim light will not attract *A. vagus* vertically downwards. 'Cages' set up as in fig. 5B.

'Cage'	Reservoir (R)	Main Cage (M)	Light Trap (L)	
—	2	17	0	% entering L.
Fig. 5B	7	40	0	
TOTAL ..	9	57	0	0

It is suggested that figures 4 and 5 and Tables III, IV and V prove that dim light exerts an attraction. This attraction can divert *A. vagus* from upward flight to an extent which depends upon the degree of opposition between the two forces involved (negative geotropism and positive phototropism), the extent being determined by the position of the light source. Attraction to dim light is not strong enough to cause *A. vagus* to fly vertically downwards.

Miscellaneous observations.

The following miscellaneous observations were also made :—

If the reservoir is not blacked out, the response to light quite overwhelms the tendency to upward flight, and hardly any mosquitos leave the reservoir. The result of one experiment when R was not blacked out was :—R 99, M 4, L 4. 'Cage' set up as in fig. 2B, though not in dark-room.

The experiment cannot be performed by day, as the mosquitos remain quiescent and will not leave the reservoir, even though it is blacked out. The result of one experiment made by day between 9.10 a.m. and 3.30 p.m. was :—R 100, M 16, L 3. 'Cage' set up as in fig. 2B, but not in dark-room and R blacked out.

Anopheles maculatus Theo., under these conditions, does not respond to light as readily as *A. vagus*. With the 'cage' set up as in fig. 4B, about 90 per cent. of *A. vagus* enter the light trap (Table III), as against about 50 per cent. of *A. maculatus*; see Table VI.

TABLE VI.

Experiments showing that attraction of *A. maculatus* to dim light is less than that of *A. vagus*. Compare Table III. 'Cages' set up as in fig. 4B.

'Cage'	Reservoir (R)	Main Cage (M)	Light Trap (L)	
—	12	25	20	% entering L.
—	9	12	33	
—	9	15	25	
—	9	8	30	
TOTAL ..	39	60	108	52

If *Aedes albopictus* (Skuse) is placed in the darkened reservoir by day, very few leave it; R 180, M 11, L 6. 'Cage' as in fig. 2B, but not in dark-room.

The mosquitos would very seldom take a blood meal. In the earlier experiments a rabbit or guinea pig was placed in the main cage, but in twenty such experiments, involving about five hundred mosquitos, only five were found to have fed on the animal. The inference is that the conditions were still too artificial to permit the mosquitos to behave quite normally, and their movements (negatively geotropic and positively phototropic) were largely attempts to escape.

Discussion.

When the experiments were commenced it was hoped that the mosquitos would be attracted from the reservoir into the main cage by the odour of the guinea pig or rabbit in the latter, and that after feeding they would rest on the walls of the main cage before making their way into the light trap, probably about dawn, thus behaving much as they would in nature. However, it was soon apparent that they left the darkened reservoir whether there was an animal present or not, and it looked as if most of those in the light trap entered shortly after dark. Observation confirmed the finding of Muirhead Thomson and others that there is a period of great

activity at dusk, and it seems probable that the movements giving rise to the distribution of mosquitos in reservoir, main cage and light trap, observed the next morning, mostly took place during this period of dusk activity. The kills obtained when the panels were treated with insecticides suggest that, nevertheless, most of the mosquitos at least touched the walls of the main cage before entering the light trap although, during the first few weeks after application of the insecticide to the panels, a proportion of the deaths was probably due to fumigation (see p. 772). Several observations made after dark by partially sliding out one of the panels showed mosquitos resting on the walls (panels).

While *A. vagus* failed to behave in this apparatus as they would when entering a house or cattle shed, the apparatus did provide a means of allowing mosquitos to expose themselves to a film of residual insecticide and then to escape from it. Inasmuch as the period spent in resting in the main cage was probably less than it would have been if the mosquitos had fed on the animal, tests of insecticides by this method were probably severe; the results of these tests are given later.

It may be that if one were to use a species of *Anopheles* which will mate in captivity and which can be maintained in a laboratory colony, behaviour would be quite natural, and in that case it might be justifiable to infer from the laboratory tests what the performance of an insecticide would be against that species of *Anopheles* in the field, which is seldom possible at present.

B. THE RESULTS OF TESTS WITH WETTABLE POWDERS OF DDT, BHC AND CHLORDANE ON PLYWOOD PANELS.

The apparatus employed in these experiments and the method of use have been described above. Each test consisted of two experiments performed on successive nights. The experimental and control cages were placed side by side with their light traps equidistant from the source of illumination and with their reservoirs blacked out (see fig. 6).

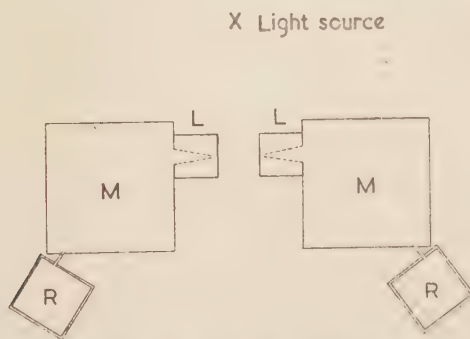


Fig. 6.—Diagrammatic side view of the cages set up for an experiment. R, reservoir; M, main cage; L, light trap. The double line round R indicates that it was blacked out. The light source was a 15 watt frosted electric lamp, partially blacked out, hanging about 3 feet above the light traps.

The reservoir of each cage contained about 50 *A. vagus*, male and female. On the second night the cages were transposed, that which had been on the left being placed on the right and *vice versa*. A separate main cage and light trap were employed for each insecticide. Temperatures varied between about 73° and 85°F.

In these tests the toxicity and residual effect of wettable powders of DDT, BHC and Chlordane on plywood were compared. The insecticides were mixed with 100 cc. of water and applied with a paintbrush to the plywood panels 24 hours before the first test. Between tests the panels were stored upright in a rack on a verandah where there was free circulation of air, but no exposure to sun or rain. DDT (Stafford Allen, 33 per cent.) was applied at 200 mg. per sq. ft. ; BHC (I.C.I., Gammexane P. 520) at 40 mg. gamma isomer per sq. ft. ; and Chlordane (Julius Hyman, 50 per cent.) at 200 mg. per sq. ft.

Results from the main Series of Tests.

The principal results from these tests are summarised in Tables VII, VIII and IX, and the total overnight kills adjusted by Abbott's formula (Abbott, 1925), so as to allow for the mortality in the Controls, are shown in Table X and graphically in fig. 7.

TABLE VII. DDT.

Results of tests of DDT wettable powder at 200 mg. DDT per sq. ft. Mortality next morning among adult *A. vagus* exposed to treated panels for unknown periods overnight.

Weeks after treatment	DDT CAGE										CONTROL CAGE		
	No. used	Reservoir		Main Cage		Light Trap		Total % dead		% entering light trap	No. used	Total % dead	% entering light trap
		Alive	Dead	Alive	Dead	Alive	Dead	Both sexes	♀ only				
0	97	0	9	0	31	13	44	87	95	59	94	13	85
1	100	0	4	0	44	14	38	86	90	52	100	9	92
2	118	0	7	1	27	40	43	65	74	70	111	12	85
5	96	0	3	0	13	45	35	53	57	83	96	17	95
7	111	1	2	0	24	61	23	44	58	76	102	18	91
9	96	1	7	0	24	43	21	54	50	67	86	21	94
11	102	0	7	0	17	50	28	51	59	76	91	14	94
16	118	0	2	0	21	65	30	45	58	81	104	15	88

TABLE VIII. BHC.

Results of tests of BHC wettable powder at 40 mg. gamma isomer per sq. ft. Mortality next morning among adult *A. vagus* exposed to treated panels for unknown periods overnight.

Weeks after treatment	BHC CAGE										CONTROL CAGE		
	No. used	Reservoir		Main Cage		Light Trap		Total % dead		% entering light trap	No. used	Total % dead	% entering light trap
		Alive	Dead	Alive	Dead	Alive	Dead	Both sexes	♀ only				
0	104	0	49	0	34	0	21	100	100	20	95	21	94
1	99	0	20	0	24	0	55	100	100	56	98	10	81
2	100	1	11	0	32	8	48	91	96	56	108	7	86
5	100	3	6	0	32	34	25	63	64	59	100	23	85
7	99	0	8	0	12	59	20	40	36	80	95	20	93
9	104	0	6	0	5	63	30	39	42	89	99	12	97
11	95	0	6	0	7	68	14	28	26	86	98	9	96
16	103	0	2	0	6	81	14	21	22	92	94	7	96

TABLE IX. CHLORDANE.

Results of tests of Chlordane wettable powder at 200 mg. Chlordane per sq. ft. Mortality next morning amongst adult *A. vagus* exposed to treated panels for unknown periods overnight.

Weeks after treat- ment	CHLORDANE CAGE										CONTROL GAGE		
	No. used	Reservoir		Main Cage		Light Trap		Total % dead		% entering light trap	No. used	Total % dead	% entering light trap
		Alive	Dead	Alive	Dead	Alive	Dead	Both sexes	♀ only				
0	90	2	7	0	59	0	22	98	100	24	68	19	75
1	99	1	10	0	35	5	48	94	96	54	103	16	88
2	98	0	8	0	11	25	54	74	82	81	97	19	90
5	93	1	7	0	5	62	18	32	33	86	89	8	94
7	100	0	4	0	0	73	23	27	40	96	100	10	95
9	103	1	1	0	3	80	18	21	22	95	109	11	94
14	103	2	1	0	5	85	10	15	17	92	100	10	93

TABLE X.

Total overnight kills per cent. (Corrected.)

Treatment	Weeks after treatment									
	0	1	2	5	7	9	11	14	16	
DDT	85	85	60	43	32	42	43	—	35	
BHC	100	100	90	52	25	31	21	—	15	
Chlordane ..	98	93	68	26	19	11	—	6	—	

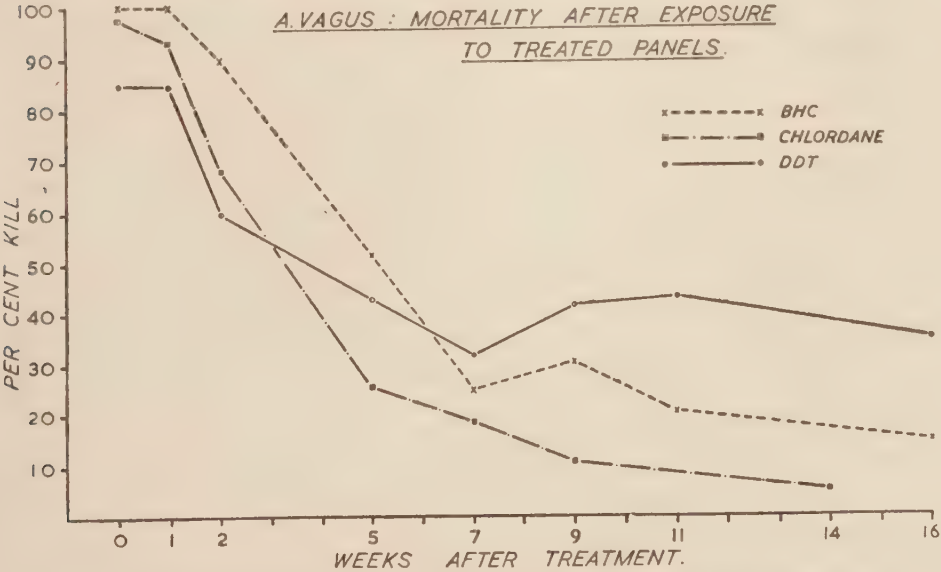


Fig. 7.—Graphic representation of the figures in Table X, showing total overnight kill of *Anopheles vagus*, after exposure to plywood panels treated with wettable powders of insecticides. DDT, 200 mg. per sq. ft.; BHC, 40 mg. gamma isomer per sq. ft.; Chlordane, 200 mg. per sq. ft.

The results are in good general agreement with those of other workers, such as Fay & others (1947) and Gahan & others (1948). Fig. 7 shows that the order of residual effectiveness is DDT, BHC, Chlordane, and that the order of toxicity when fresh is BHC, Chlordane, DDT. Table X shows that the BHC when a week old caused 100 per cent. mortality overnight. DDT never bettered 85 per cent., but whereas after 16 weeks it still killed 35 per cent. of the mosquitos, the kill from BHC had dropped below 30 per cent. by the 7th week, and that from Chlordane by the 5th week.

The Tables show that as the potency of each insecticide fell off the percentage of mosquitos living long enough to enter the light trap increased, and the mortality among them decreased; this is what happens in field experiments with treated window trap huts (Wharton, in the press). The percentage entering the light trap in each of the control series remained fairly steady between 80 and 100 per cent., with an average of 90 or 91 per cent. With a single exception (Table VII) no live mosquitos were found in the main cages in the morning, except in the control.

Anopheles maculatus. This species, which is the principal vector of malaria in Malaya, was tested against DDT and BHC and high kills were obtained, probably largely due to the weaker response to light compared with *A. vagus* (see p. 766), causing fewer to escape into the light trap. Tested against DDT when the film was 6 weeks old the corrected overnight kill of *maculatus* was 90 per cent.; of *vagus* in the fifth week 43 per cent. The corresponding kills with BHC were: *maculatus*, eighth week, 87 per cent.; *vagus*, seventh week, 25 per cent.

Delayed Mortality.

Survivors found in the light trap in the morning were placed in lamp chimneys with water and raisins, and the mortality amongst them during the next 48 hours was noted, and compared with that of a sample from the light trap of the control cage. The results are shown in Table XI.

TABLE XI.

A. vagus. Death rate among survivors from light traps 24 and 48 hours after exposure to treated and untreated panels.

Insecticide	Weeks after treatment	EXPERIMENTAL CAGE					CONTROL CAGE				
		No. kept	Dead after				No. kept	Dead after			
			24 hrs.		48 hrs.			24 hrs.		48 hrs.	
			No.	%	No.	%		No.	%	No.	%
DDT .. {	0-5	66	15	23	33	50	117	0	0	6	5
	7-16	219	13	6	31	14	96	0	0	0	0
BHC .. {	0-5	42	4	10	15	36	110	0	0	11	10
	7-11	190	9	5	20	10	72	0	0	0	0
Chlordane .. {	0-5	92	1	1	3	3	91	0	0	0	0
	7-14	238	1	0.4	4	2	72	0	0	0	0

It will be seen that during the first five weeks after treatment, DDT and BHC continued to cause considerable mortality for at least 48 hours after the mosquitos had been exposed to them; about 50 per cent. after 48 hours with DDT. From the seventh week onwards this delayed mortality was much lower, but still appreciable. DDT probably caused a greater delayed mortality than BHC, although the differences (14 per cent. during the 0-5 weeks period, and 4 per cent. during the later period) could well have been due to chance ($P = < 0.2$ and < 0.3). Chlordane caused very little delayed mortality.

Greater Mortality among Females than Males.

It will be seen from Tables VII, VIII and IX that females seem to have been more susceptible to the insecticides, especially DDT and Chlordane, than the males, but there is also a slightly higher mortality among females in the controls. A comparison of the mortalities among males and females in the controls and in the DDT tests is given in Table XII.

TABLE XII.

Comparison of mortality in males and females of *A. vagus*.

Treatment	Males			Females			Difference %
	No. used	No. dead	% dead	No. used	No. dead	% dead	
Controls ..	493	60	12	470	75	16	4
DDT	403	209	52	435	295	68	16

The difference in mortalities in the controls is 4 per cent. ($P = < 0.1$); in the DDT tests it is 16 per cent. ($P = < 0.01$). In other words the probability that the greater mortality among females in the DDT tests is due to chance is less than one per cent., but in the controls this probability is nearly 10 per cent.

It is not clear why females should have been more susceptible to the insecticides than males. The proportions of both sexes entering the light traps were the same, as Table XIII shows, but it may be that the males tended to enter more rapidly, spending less time in contact with the treated panels of the main cage. It does not seem likely, in view of the results obtained by other workers, that males were innately less susceptible than females. David & Bracey (1946) concluded that male *Aedes aegypti* (L.) were innately more susceptible to DDT or pyrethrins than females, though if the flying insects were subjected to a spray containing DDT, males were less susceptible than females. They showed that this was due to the females flying more in these sprays than males; if motionless insects were sprayed the males suffered greater mortality. Cutkomp (1947) found that male *Anopheles quadrimaculatus* Say were more rapidly knocked down by residual films of DDT, BHC or Chlordane than females.

TABLE XIII.

Relative numbers of males and females of *A. vagus* entering the light traps.

Sex	All Controls (8 tests)		DDT (4 tests)	
	No. used	% entering light trap	No. used	% entering light trap
Males	398	89	190	67
Females ..	394	89	221	66

Fumigation.

The heavy mortality in the BHC reservoir during the first two tests (Table VIII) suggested that fumigation had occurred. BHC is well known to exert a fumigant effect, but since this effect has also been demonstrated for Chlordane, *e.g.*, by Dustan & others (1947), it was rather surprising to find that there was no significant difference between the mortalities in the Chlordane and Control reservoirs. Separate experiments were therefore made to investigate this question. The usual arrangement of cages was employed (fig. 6), but mosquito gauze was placed over the lower end of the tube connecting the reservoir to the main cage so that the mosquitos in the reservoir were confined there. A bowl of 4th instar *A. vagus* larvae was placed in the main cage and a lamp chimney with adults, both with covers supported just above them so that no solid particles could fall inside. Adults were also placed in the light trap. Each experiment consisted as usual of an experimental cage (with panels freshly treated with wettable powders at the same dosage as in the main series of tests) and a control cage, and was performed on two successive nights in the cool and humid room of the insectarium in which the main series of tests was performed, and in which there is little movement of air.* The results are summarised in Tables XIV and XV.

TABLE XIV.

Mortality of *A. vagus* adults caused apparently by vapour from films of insecticides not more than 48 hours old (wettable powders).

Treatment	Reservoir		Main Cage		Light Trap	
	Alive	Dead	Alive	Dead	Alive	Dead
Gamma BHC 40 mg/ft.* ..	9	40	0	30	0	58
Chlordane 200 mg/ft.*	29	24	0	20	0	30
Control (Aver. of 3) ..	48	4	21	3 ..	30	7

TABLE XV.

Mortality of *A. vagus* larvae caused apparently by vapour of insecticides (wettable powders).

Treatment	Alive	Dead
BHC	0	30
Chlordane	9	21
Control (Aver. of 3) ..	30	0

Evidently fresh films of BHC and Chlordane have a fumigant effect to which *A. vagus* is very susceptible. None of the adult mosquitos in the experimental main cages or light traps lived, and a variable proportion of those in the reservoirs was killed. The mortality amongst the larvae, together with that amongst the adults confined in the reservoirs, shows BHC to have the stronger fumigant effect. DDT was also tested and a discussion of the results will be found on page 774.

* During June and July, 1949, when these experiments were performed, temperature and humidity in this room were as follows:—

	June	July
Mean maximum temperature °F.	85	83
Mean minimum " " "	78	77
Highest maximum " " "	86	85
Lowest minimum " " "	73	76
Rel. humidity % at 9 a.m. " "	80	78

From about 11 p.m. to 8 a.m., relative humidity is almost continuously over 90 per cent. throughout the year in Kuala Lumpur.

Repellency.

Since Chlordane seemed to exert a considerable fumigant effect, but unlike BHC had not caused any excess deaths in the reservoir in the early part of the first series of tests (Table IX), it seemed possible that BHC might also be exerting a repellent effect, tending to prevent the mosquitos from leaving the reservoir with the result that they were killed there by fumigation. Admittedly a sufficiently powerful fumigant action by BHC could also account for these results but certain observations from field experiments (Wharton, in the press) also suggested that BHC might have some repellent effect at a distance and some simple experiments were therefore carried out. The principle was to count the number of *A. vagus* adults resting on one mosquito gauze side of a small cage (one of the reservoir cages), and then to bring up close to it on the outside, a treated plywood panel, and recount the number of resting mosquitos after 15 minutes. In practice each test was made with two cages (one experimental, one control) placed equidistant from a dimly lighted window. The panels, one of which was treated with insecticide and the other left untreated, were one quarter square foot in area, slightly smaller than the gauze sides of the cages. Counts were made on three sides of the cages in succession, omitting the fourth side which faced towards the window, and testing the corresponding sides of each cage simultaneously; the sum of the counts on the three sides formed one test. After making the initial count on any side, the panel, held in a retort stand, was brought up slowly to a position about one inch from, and parallel to, the side under observation and kept there for 15 minutes. Two fresh films of BHC were tested in this manner, one was a wettable powder, and the other a deposit from a benzene solution; citronella oil was also tested for comparison, since it is a well known repellent with a greater action at a distance than most others (Christophers, 1947). The results are summarised in Table XVI.

TABLE XVI.

Results of repellency tests with adult *A. vagus*.

Treatment	No. of tests	Number of mosquitos resting on sides of cages			Mortality 24 hrs. after exposure	
		At start S.	At finish F.	F/S	No.	Per cent.
BHC	2	45	15	0.33	97/114	81
Citronella ..	1	36	8	0.22	4/59	7
Control ..	5	170	177	1.04	11/170	6

Explanation.—BHC: 40 mg. gamma isomer per sq. ft., wettable powder, and 80 mg. benzene solution. Citronella: 0.25 cc. applied in drops to the panel. Control: 5 tests with 3 cages, two of which were used twice. Cages usually contained about 50 mosquitos, but only a proportion of these rested on the sides where counts were made, so that the figures in columns S and F are considerably less (except in the control for the reason given) than the total numbers employed, which appear in the first column of mortality, i.e., 114, 59, 170.

This table shows the marked repellent effect of citronella and BHC*, but whilst the mortality 24 hours after exposure to citronella is the same as in the controls, about 7 per cent., the mortality after exposure to BHC is high, 81 per cent. It seems

* The test shows that the probability that the ratio F/S for BHC could be due to chance is less than one in 1,000.

that if there is enough BHC vapour to cause repellence, it is also enough to cause considerable mortality. There is no reason to doubt that the figures for BHC do indicate a repellent effect, and not mere 'knock-down', for this did not occur until some time after the 15 minutes' exposure; although a few mosquitos showed some signs of distress towards the end of the exposure. Furthermore, it was observed that there was a strong tendency for the mosquitos to move to the side of the cage farthest from the BHC treated panel, the average number on this side in one test rose from 15 at the start to 37 at the end of the exposure. Chlordane was not tested for repellency, as time was short and the main series of tests had shown that it was unlikely to be used in residual house spraying for mosquito control.

DDT was tested for any fumigant or repellent effects, in the same manner and at the same time as BHC and Chlordane, and unexpectedly gave positive results. In the fumigation tests the wettable powder of DDT used in the main series of tests (Table VII) gave a kill of adults and larvae not very much lower than that caused by Chlordane. In the repellency tests there was some evidence of a repellent effect, and subsequent mortality was near 50 per cent. Since other workers had been unable to demonstrate any fumigant effect with DDT, these results clearly needed confirmation. Dustan & others (1947), using the plant bug, *Oncopeltus fasciatus* (Dall.) found no fumigant effect with DDT, though it was strong with BHC and pronounced with Chlordane. Hoffman and Lindquist (1949) found no fumigant effect of DDT on *Musca*, though it was readily shown with BHC, Chlordane, Toxaphene and Parathion. The experiments with DDT were therefore repeated, using the same wettable powder and taking care to avoid any likely sources of error. Blank trials were made with experimental and control cages to see that both were similar, before treating the panels for the experimental cage. Repetition gave the same results as before, though the kill in the fumigation test was a little less. A brief account of the repellency tests has already been published (Field, 1950).

The next step was to try pure DDT instead of wettable powders. A sample of pure *para para* DDT was received from London, kindly sent by Professor Buxton and Dr. Busvine, and trials for action at a distance were started again. This time the wettable powder of DDT gave no kill at all, nor did the pure DDT, though BHC behaved as before. Various experiments were tried and a more artificial but more easily repeatable technique was evolved. Glass rings about 7 cms. high and 9 cms. in diameter were stood upon a sheet of plate glass treated with films of insecticide. Inside the bottom of each glass ring was fitted a cardboard ring about 1-2 cms. high over which was stretched mosquito netting; this prevented mosquitos from coming in contact with the treated glass plate. Five mosquitos were confined in each ring and the tops were closed with mosquito netting on which was put a little damp cotton wool and split raisins. On top of each ring, enclosing the cotton wool and raisins, was placed an inverted half petri dish of the same diameter as the ring, thus converting the ring into a closed chamber. The sheet of plate glass was treated in strips, each large enough to accommodate five glass rings. In this way several insecticides could be tested simultaneously for fumigant effect, exposing 20 mosquitos to each. Earlier attempts to employ this technique with lamp chimneys were not satisfactory as there were insufficient surfaces suitable for the mosquitos to rest on, so that they kept flying and disturbing each other with a consequent high death rate in the controls.

Using the rings, adult *A. vagus* 36 hours old were tested against various samples of DDT. The rings were placed upon the treated strips on the glass sheet at 4 p.m., and the number of mosquitos alive and dead counted the next morning. DDT at 400 mg. per sq. ft. was applied as benzene solutions, when the control strip was treated with plain benzene, or as water suspensions of wettable powders. Gamma BHC at 5 mg. per sq. ft. was applied as a benzene solution. The results are shown in Table XVII.

TABLE XVII.

Results of fumigation tests with DDT (400 mg/sq. ft.) and gamma BHC (5 mg/sq. ft.) using adult *Anopheles vagus* confined in glass rings over a treated glass plate. Twenty mosquitos per test. Numbers dead and alive after about 17 hours.

Treatment	No. of tests	No. dead	No. alive
Control	4	3	77
Pure <i>p, p'</i> DDT	4	5	75
83% <i>p, p'</i> DDT (Geigy)	3	3	57
33% wettable powder DDT (Stafford Allen)	4	2	78
25% wettable powder DDT (I.C.I.)	3	60	0
Pure gamma BHC	1	20	0

BHC, pure gamma, or wettable powder, gave a complete kill in this and earlier tests, and knock-down was usually complete in about one hour or less. In view of the failure of the DDT 33 per cent. wettable powder to cause any mortality in this test (and in the repellency test when repeated a third time), the published results (Field, 1950*) and those reported here, showing a fumigant and repellent effect with this powder, are best discounted, although it is very difficult to see how the results can have been false. That these powders may have a fumigant effect is shown by the complete kill obtained with the 25 per cent. application (Table XVII), but any such effect seems unlikely to be due to the *p, p'* DDT content of the powder since pure *p, p'* DDT caused no mortality.

Lack of time and staff prevented this enquiry from being continued, but further investigation seems desirable. A number of field workers have suspected a repellent effect with DDT, and it seems worth while to try to find out if commercial preparations of DDT are really liable to be repellent to some degree. Busvine and Kennedy (1949) found evidence of DDT and BHC driving cockroaches out of treated rooms into adjacent ones which were previously uninfested, but it is not clear whether this was due to repellency in the sense in which the term is employed here, i.e., effect at a distance, or to avoidance of contact with a source of irritation. Christophers did not find DDT repellent to *Aedes aegypti*. Gabaldon (1949) and Gebert (1948) believe that DDT in houses has a repellent effect on Anophelines (*A. darlingi* Root in Venezuela, and *A. funestus* Giles in Mauritius respectively). Thomson (1950) also found what resembled a repellent effect of DDT upon *A. funestus*. When he treated a trap hut with a heavy dose of a wettable powder of DDT, *A. gambiae* continued to enter as freely as before, but the numbers of *funestus* entering dropped from an average of 8 per night to less than 1 per night, while the number entering an untreated control hut rose. Thomson suggests that this might not be due to repellence at a distance but to contact by the mosquitos with small amounts of DDT whilst attempting to enter the hut, in other words an irritant effect. Bertram (1950) speaks of a repellent effect of light doses of DDT on *A. minimus* Theo., but it seems more likely to be an irritant effect as with *A. gambiae* (Thomson, 1947, 1950). Caution is needed in using the term 'repellent' which is probably best confined to describing effects at a distance. The existence of an irritant effect on mosquitos after contact with DDT, causing them to seek to escape to the light, has been accepted since Kennedy (1947) demonstrated it in laboratory experiments. In the field, Thomson (1947) found an apparent irritant effect of DDT upon *A. gambiae*, and Wharton and Reid (1950) found the same for *Culex fatigans* Wied. Bertram's figures suggest that *A. minimus* may be irritated

* In order to secure early publication, Dr. Field kindly reported these results for me in a letter to the *Trans. R. Soc. trop. Med. Hyg.* He is not responsible for any errors there may be: these are mine.—J.A.R.

but not killed by small doses of DDT, though the lack of figures from a control hut makes confirmation desirable. But the existence under field conditions of repellence at a distance with commercial BHC or DDT requires proof. It probably exists with fresh BHC for the effect was very marked in laboratory experiments (Table XVI), whilst in field experiments with trap huts and wettable powders (Wharton, in the press) there was a sharp reduction in the numbers of *C. fatigans* entering for a week after treatment with BHC, and a smaller reduction lasting three weeks in the numbers of *A. maculatus*. The numbers of *A. maculatus* were also reduced during the first three weeks after treatment with DDT, though this did not effect the number of *C. fatigans* which are resistant to DDT (cf. *A. gambiae*). It seems clear that the exact effects obtained with these insecticides depend very much upon the species of mosquito.

Summary.

Three interconnecting cages, the largest of which was about 2 feet square, were used in experiments with *Anopheles vagus* bred from wild-caught larvae.

Variations in the arrangement of these cages showed that :

1. The mosquitos are quiescent by day, if not disturbed, whether kept in the light or in darkness.
2. If they are in complete darkness by night, as in a photographic darkroom, they fly vertically upwards. They will readily escape from a cage about one foot square, with a hole half an inch in diameter in the top, in their continued attempts to fly upwards.
3. They are attracted by a dim light at night and will fly sideways or to a lesser extent slightly downwards, in opposition to the tendency to vertical upward flight. In this way they can pass through openings which they would miss if in complete darkness. Light will not cause them to fly vertically downwards.
4. *Anopheles maculatus* is less responsive to light under these conditions, than *A. vagus*.

Using *A. vagus*, tests were made of the toxicity and residual effect of DDT, BHC and Chlordane as wettable powders on plywood panels. The order of toxicity when fresh was found to be BHC, Chlordane, DDT ; the order of the residual effectiveness was DDT, BHC, Chlordane.

A considerable mortality occurred amongst mosquitos surviving the overnight tests with DDT and BHC, if they were kept for a further 48 hours.

A larger proportion of females was killed than of males, especially by DDT and Chlordane.

BHC was shown to have a marked repellent effect, comparable with that of citronella, but whereas citronella repelled without killing, BHC repelled, but also caused a high subsequent mortality.

A. maculatus was tested once against DDT, and once against BHC in the main series of tests, and was much more readily killed than *A. vagus*, presumably because of its weaker response to light causing it to remain longer in the main cage in contact with the treated panels.

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References.

- ABBOTT, W. S. (1925). A method of computing the effectiveness of an insecticide. — J. econ. Ent., **18**, pp. 265–267.
- BERTRAM, D. M. (1950). Malaria control by residual insecticides. — Brit. med. J., May 20th, 1950, p. 1,200.
- BUSVINE, J. R. & KENNEDY, J. S. (1949). Experiments with insecticidal smokes for indoor use. — Ann. appl. Biol., **36**, pp. 76–85.
- CHRISTOPHERS, S. R. (1947). Mosquito repellents. — J. Hyg., **45**, pp. 176–231.
- CUTKOMP, L. K. (1947). Residual sprays to control *Anopheles quadrimaculatus*. — J. econ. Ent., **40**, pp. 328–333.
- DAVID, W. A. L. & BRACEY, P. (1946). Factors influencing the interaction of insecticidal mists on flying insects. Part III. Biological factors. — Bull. ent. Res., **37**, pp. 177–190.
- DUSTAN, G. G., ARMSTRONG, T. & PUTNAM, W. L. (1947). The influence of air currents on the insecticidal action of DDT, benzene hexachloride, Hercules Toxicant 3956, and Velsicol 1068. — Canad. Ent., **79**, pp. 45–50.
- FAY, R. W., COLE, E. L. & BUCKNER, A. J. (1947). Comparative residual effectiveness of organic insecticides against house flies and malaria mosquitos. — J. econ. Ent., **40**, pp. 635–640.
- FIELD, J. W. (1950). Fumigant and repellent effects of BHC (Gammexane) and DDT upon *Anopheles*. — Trans. R. Soc. trop. Med. Hyg., **43**, pp. 547–548.
- GABALDON, A. (1949). The nation-wide campaign against malaria in Venezuela. — Trans. R. Soc. trop. Med. Hyg., **43**, pp. 113–160.
- GAHAN, J. B., GILBERT, I. H., PEFFLEY, R. L. & WILSON, H. G. (1948). Comparative toxicity of four chlorinated organic compounds to mosquitos, house flies and cockroaches. — J. econ. Ent., **41**, pp. 795–801.
- GEBERT, S. (1948). Notes on certain aspects of the action of DDT residual sprays, and on the partial treatment of dwellings as a means of anti-anopheline protection. — Trans. R. Soc. trop. Med. Hyg., **42**, pp. 295–297.
- HOFFMAN, R. A. & LINDQUIST, A. W. (1949). Fumigating properties of several new insecticides. — J. econ. Ent., **42**, pp. 436–438.
- KENNEDY, J. S. (1947). The excitant and repellent effects on mosquitos of sub-lethal contacts with DDT. — Bull. ent. Res., **37**, pp. 593–607.
- THOMSON, R. C. M. (1947). The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. — Bull. ent. Res., **38**, pp. 449–464.
- THOMSON, R. C. M. (1948). Studies on *Anopheles gambiae* and *A. melas* in and around Lagos. — Bull. ent. Res., **38**, pp. 527–558.
- THOMSON, R. C. M. (1950). DDT and Gammexane as residual insecticides against *Anopheles gambiae* in African houses. — Trans. R. Soc. trop. Med. Hyg., **43**, pp. 401–412.
- WHARTON, R. H. & REID, J. A. (1950). DDT and Gammexane as residual insecticides against *Anopheles maculatus* in Malaya. — Nature, **165**, pp. 28–29.
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